JOURNAL OF AGRICULTURAL RESEARCH

VOL. 76 WASHINGTON, D. C., JUNE 15, 1948 NO. 12

EFFECT OF DIFFERENT VARIETIES AND AGES OF SORGHUM ON THE BIOLOGY OF THE CHINCH BUG[1]

By R. G. DAHMS[2]

Associate Entomologist, Division of Cereal and Forage Insect Investigations, Agricultural Research Administration, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, and associate professor of research in entomology, Oklahoma Agricultural Experiment Station

INTRODUCTION

Measures generally recommended for the control of the chinch bug (*Blissus leucopterus* (Say)) include the construction of barriers to protect susceptible crops, the planting of nonhost crops, and the separation of small grains from corn and sorghums. In the southern part of the infested area in Oklahoma the principal method advocated against this pest, namely, the construction of barriers, is not reliable, because by the time the grains ripen, the insects have usually reached the adult stage and they migrate to corn and sorghums by flight rather than by crawling. A partial solution of the problem of chinch bug control in this area was suggested by the recent development of varieties of plants resistant to insect attack, especially since the many varieties of sorghums growing in the nursery plots at Lawton, Okla., showed different degrees of resistance (*10, 11*).[3] As a step toward determining the cause of this resistance, the biology of the chinch bug has been studied with especial reference to the effect of the sorghum variety on longevity, fecundity, and rate of egg deposition of the adults, and on the mortality and rate of development of the nymphs. The comparative tolerance of certain varieties to injury caused by the feeding of the adults and the host preferences of adults have also been studied. The data presented in this paper are based upon studies made at Lawton during 1936 and 1937.

REVIEW OF LITERATURE

The effect of the host on the biology of an insect has been mentioned by various investigators. McColloch and Salmon (*6*) showed that the

[1] Received for publication October 29, 1947. Cooperative investigation between the Oklahoma Agricultural Experiment Station, the Bureau of Plant Industry, Soils, and Agricultural Engineering, and the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture. Abstract of a portion of a thesis submitted to the Kansas State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] The author is indebted to the following for valued assistance in the execution of the work and the preparation of the manuscript: F. A. Fenton, of the Oklahoma Agricultural Experiment Station; W. M. Osborn, J. H. Martin, and R. O. Snelling, of the Bureau of Plant Industry, Soils, and Agricultural Engineering; and P. N. Annand and C. M. Packard, of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.

[3] Italic numbers in parentheses refer to literature cited, p. 287.

hessian fly (*Phytophaga destructor* (Say)) laid fewer eggs on four varieties of durum wheat than on any of the winter wheats studied. Hodge (*4*) found a wide range of mortality when the grasshopper *Melanoplus differentialis* (Thos.) was reared from egg to adult on different hosts, and Seamans and McMillan (*9*) obtained similar results with the pale western cutworm (*Agrotis orthogonia* Morr.). Wadley (*13*) noted that the mortality of the green bug (*Toxoptera graminum* (Rond.)) was higher on durum wheat than on common wheat. Isely (*5*) reported that the duration of the larval stage and the fecundity of the bollworm (*Heliothis armigera* (Hbn.)) varied considerably when the insect was reared on different host plants. Blanchard and Dudley (*1*) found alfalfa plants on which the pea aphid (*Macrosiphum pisi* (Kltb.)) was unable to maintain a population. Painter (*8*) also found considerable difference in the reproductive ability of the pea aphid on different varieties of alfalfa and even on flowering and vegetative branches of the same plant. According to DeLong and Jones (*3*), the gooseberry aphid (*Kakimia houghtonensis* (Troop)) could not maintain a population on certain plants of the Houghton variety. Winter (*14*) showed that the raspberry aphid (*Amphorophora rubi* (Kltb.)) reproduced much more slowly on some varieties than on others.

Snelling and coworkers (*10*) showed that different varieties of sorghums react differently to heavy infestations of chinch bugs in Oklahoma. Dahms et al. (*2, 7*) found considerable difference in the rate of development and mortality of chinch bug nymphs as well as in the length of life and number of eggs laid by females when fed different varieties of sorghums and other host plants.

VARIETIES OF SORGHUM USED

The varieties of sorghum chosen for these tests were those representatives of the four major divisions—milos, feteritas, kafirs, and sorgos—that had shown the greatest or the least resistance under field conditions. Since Vinall, Stephens, and Martin (*12*) have published descriptions of most of them, only the most important characters of each variety are given here.

Kansas Orange sorgo × Dwarf Yellow milo (Ks. 24136) is the most resistant selection tested under field conditions. It produces satisfactory yields of grain, but has a buff-colored seed, which is an undesirable characteristic. It is not leafy, has dry, pithy stalks, and is therefore unsatisfactory for forage. This variety has not been distributed to farmers.

Atlas sorgo (C. I. 899) is a cross between Blackhull kafir and Sourless sorgo. It is highly resistant to chinch bugs and well adapted to Oklahoma conditions. This dual-purpose variety is leafy and has white palatable grain and sweet, juicy stalks, which are resistant to lodging.

Kansas Orange (F. C. 9108) is a sorgo that has been grown for many years in eastern Kansas. It shows a great deal of resistance to the chinch bug, but is less desirable than Atlas because of its brown, bitter seed and the tendency of its stalks to lodge.

Blackhull kafir (C. I. 71) is widely grown in Oklahoma, Texas, and Kansas, and has been popular for many years. Its origin is unde-

termined. Sharon kafir (C. I. 813) is a selection from Blackhull and is similar to it. Sharon was used in these tests because it is probably one of the parents of Wheatland. Both Blackhull and Sharon kafir are rather resistant to chinch bug.

Feterita (C. I. 182), an African introduction, formerly was an important variety of grain sorghum in Texas, Oklahoma, and Kansas. It is moderately susceptible in its reaction to chinch bugs under field conditions, but because of its earliness it often escapes serious damage.

Dwarf Yellow milo (C. I. 332) is a popular grain sorghum outside the chinch bug-infested territory, but is so susceptible to chinch bug attack that it cannot be grown in sections of the State where chinch bug is prevalent.

Wheatland (C. I. 918) is a cross between kafir (probably Sharon) and Dwarf Yellow milo. It formerly was popular in northwestern Oklahoma and southwestern Kansas as a grain sorghum suitable for harvesting with a combine harvester, but, like Dwarf Yellow milo, it is so susceptible to chinch bugs that it is not grown where they are abundant.

Honey is a tall, juicy sorgo, which is grown for sirup and silage in the Southeastern States. Although not listed in table 1, it is known to be susceptible to the chinch bug.

COMPARATIVE VARIETAL SUSCEPTIBILITY UNDER FIELD CONDITIONS

The effects of chinch bug infestations on several sorghum varieties in the field, as reported by Snelling and coworkers (*10*), are presented in table 1. The results for 1931 are not included because the infestation that year was not heavy enough to show clear-cut varietal differences. All these observations were made on plants growing in the field under natural conditions.

TABLE 1.—*Percentage of plants of several sorghum varieties killed by chinch bugs, Lawton, Okla., 1930, 1932, 1933, and 1934* [1]

Variety	Record No.[2]	Percentage of plants killed				
		1930	1932	1933	1934	4-year average
Kansas Orange sorgo × Dwarf Yellow milo.	Ks. 24136	20	2	3	3	7
Altas sorgo	C. I. 899	20	7	20	5	13
Sharon kafir	C. I. 813	42	1	8	5	14
Kansas Orange sorgo	F. C. 9108	38	7	21	2	17
Blackhull kafir	C. I. 71	37	7	37	9	23
Feterita	C. I. 182	98	39	24	43	51
Wheatland	C. I. 918	98	47	100	100	86
Dwarf Yellow milo	C. I. 332	100	100	100	100	100
Average		56.6	26.3	39.1	33.4	38.9

[1] Data taken from Snelling et al. (*10*).
[2] Ks.—Kansas Agricultural Experiment Station number. C. I.—Accession number, Division of Cereal Crops and Diseases. F. C.—Accession number, Division of Forage Crops and Diseases.

The chinch bug infestation was of about the same intensity in 1933 and 1934, being greater in these years than in 1932 but less than in 1930. Nevertheless, the relative injury among the varieties was comparable in the four seasons. The average mortality due to chinch

bug infestation for the eight varieties ranged from 7 percent on Kansas Orange sorgo × Dwarf Yellow milo (Ks. 24136) to 100 percent on Dwarf Yellow milo. An analysis of the results given in table 1 showed that the differences between years and between varieties were both highly significant.

LABORATORY STUDIES

CONDITIONS AND METHODS

The experiments in 1936 were conducted in a laboratory where the average daily temperatures ranged from 56° to 91° F. The average temperatures during the oviposition period of overwintered, first-generation, and second-generation adults were 74.1°, 83.1°, and 72.2°, respectively, and during development of first-generation, second-generation, and third-generation nymphs they were 76.4°, 84.8°, and 73.3°.

The oviposition and nymph-rearing cage consisted of a transparent celluloid tube about 70 mm. long and 26 mm. in diameter, over one end of which a piece of cloth had been glued. Seedling plants 5 to 6 days old that had been growing in soil out of doors were used as food. Each plant was placed between the halves of a split rubber stopper with the roots extending below and the leaves above. The small end of the stopper was then inserted in a shell vial containing water so that the roots were immersed, while the larger end was fitted tightly into the open end of the celluloid tube so that the leaves were on the inside. The plants were changed daily, at which time egg counts were made. The water in the vials was changed once a week. A pair of chinch bugs was placed in each tube. The male was replaced if it died, but the experiment was discontinued at the death of the female.

EFFECT OF VARIETY ON OVIPOSITION

Oviposition records were kept on nine varieties of sorghum during 1936—the eight listed in table 1 and Honey sorgo. Ten pairs from each of the three generations of bugs were tested on each variety. Adult bugs of the overwintered generation were collected from bunch grass just before they were ready to leave their hibernating quarters. The bugs of the later generations were collected in the field when in the fifth instar, from sorghum of the same varieties on which they were to be placed in the laboratory.

The results of oviposition are given in table 2. Dwarf Yellow milo was the only variety on which all the females of the overwintered generation laid eggs. Those feeding on Atlas sorgo, feterita, and Kansas Orange sorgo laid an average of less than 1 egg per female. All 10 females of the first-generation feeding on Dwarf Yellow milo, Wheatland, and Honey sorgo laid eggs, but none of those feeding on Kansas Orange sorgo oviposited. Although 9 of the 10 females that fed on Atlas sorgo oviposited, the average number of eggs per female was only 8.9 as compared with 132.9 on Dwarf Yellow milo and 139.7 on Wheatland. On most varieties fewer eggs were laid by second-generation than by first-generation bugs. The average number of eggs laid per female for second-generation bugs ranged from 0.7 on Kansas Orange sorgo to 56.9 on Wheatland.

TABLE 2.—*Oviposition of 3 generations of chinch bugs on 9 varieties of sorghum in laboratory studies* [1]

Variety	Overwintered generation			Average temperature	First generation			Average temperature	Second generation			Average temperature	Total		
	Females laying eggs	Eggs laid per female			Females laying eggs	Eggs laid per female			Females laying eggs	Eggs laid per female			Females laying eggs	Eggs laid per female	
		Range	Average			Range	Average			Range	Average			Range	Average
	Number	*Number*	*Number*	° *F.*	*Number*	*Number*	*Number*	° *F.*	*Number*	*Number*	*Number*	° *F.*	*Number*	*Number*	*Number*
Dwarf Yellow milo	10	44–228	123.0	72.4	10	9–211	132.9	82.9	10	6–135	42.3	73.1	30	6–228	99.4
Wheatland	8	0–192	82.5	71.3	10	39–278	139.7	82.4	10	7–119	56.9	71.8	28	0–278	93.0
Honey sorgo	9	0–144	72.8	74.1	10	5–95	43.4	83.4	9	0–60	21.7	82.0	28	0–144	46.0
Blackhull kafir	9	0–25	10.8	71.9	9	0–48	18.2	83.1	7	0–109	34.7	71.8	25	0–109	21.2
Sharon kafir	4	0–101	23.8	72.4	7	0–20	7.5	82.0	6	0–72	17.7	76.4	17	0–101	16.3
Kansas Orange sorgo × Dwarf Yellow milo	2	0–26	4.9	71.4	5	0–32	7.5	80.9	6	0–49	9.4	78.7	13	0–49	7.3
Atlas sorgo	2	0–6	.8	67.1	9	0–22	8.9	82.0	1	0–21	2.1	85.1	12	0–22	3.9
Feterita	1	0–1	.1	68.2	5	0–16	4.2	81.7	2	0–7	.8	85.0	8	0–16	1.7
Kansas Orange sorgo	1	0–1	.1	67.3	0	0	0	81.7	3	0–3	.7	85.0	4	0–3	.3
Average			35.4				40.3				20.7				32.1
Difference required for significance at odds of 19 to 1															40

[1] 10 pairs of bugs were used with each host plant for each generation.

When the results for all three generations are considered, bugs feeding on Dwarf Yellow milo and Wheatland produced on an average significantly more eggs than any of the bugs feeding on the other varieties. The differences between the average number of eggs per generation were not statistically significant.

EFFECT OF VARIETY ON LONGEVITY

The longevity of the females feeding on the nine varieties for the three generations is shown in table 3. Bugs of the overwintered gen-

TABLE 3.—*Longevity of 3 generations of chinch bug females on 9 varieties of sorghum in laboratory studies*

Variety	Overwintered generation			First generation			Second generation			Total longevity	
	Average temperature	Longevity		Average temperature	Longevity		Average temperature	Longevity		Range	Average
		Range	Average		Range	Average		Range	Average		
	°F.	Days	Days	°F.	Days	Days	°F.	Days	Days	Days	Days
Wheatland	71.3	35–103	74.8	82.4	32–83	64.0	71.8	15–157	59.0	15–157	65.9
Dwarf Yellow milo	72.4	35–115	88.1	82.9	12–83	64.2	73.1	15–120	36.0	12–120	56.8
Honey sorgo	74.1	47–134	95.2	83.4	18–77	40.4	82.0	4–59	27.2	4–134	54.3
Blackhull kafir	71.9	33–111	61.0	83.1	4–83	34.3	71.8	4–157	49.4	4–157	48.2
Sharon kafir	72.4	28–115	63.9	82.0	20–51	30.8	76.4	6–87	28.0	6–115	40.9
Kansas Orange sorgo × Dwarf Yellow milo	71.4	28–105	50.8	80.9	9–34	18.8	78.7	4–73	28.0	4–105	32.5
Feterita	68.2	28–71	41.1	81.7	6–43	27.9	85.0	4–26	12.8	4–71	27.3
Atlas sorgo	67.1	28–45	35.0	82.0	4–43	24.0	85.1	5–31	18.8	4–45	25.9
Kansas Orange sorgo	67.3	28–56	35.6	81.7	6–39	15.1	85.0	4–26	13.6	4–56	21.4
Average			60.6			33.5			30.3		41.5
Difference required for significance at odds of 19 to 1											18.2

eration lived significantly longer than those of the other two generations. It should be kept in mind that the data for the longevity of overwintered bugs represents only their average longevity after they were brought into the laboratory. In most cases several days elapsed before they began laying eggs.

The average longevity of bugs of all three generations was greatest on Wheatland. Tnen in order came Dwarf Yellow milo, Honey sorgo, Blackhull kafir, Sharon kafir, Kansas Orange sorgo × Dwarf Yellow milo, feterita, Atlas sorgo, and Kansas Orange sorgo. There is a very close correlation between the number of eggs laid and the longevity of the female.

EFFECT OF VARIETY ON SIZE OF ADULTS

The relation between the size of adult chinch bugs and the variety of sorghum that they fed upon during their nymphal stage is shown in table 4. The bugs reared on the susceptible varieties, Wheatland and Dwarf Yellow milo, were the largest, and those reared on the resistant Kansas Orange were the smallest. Statistically, the differences in body length between generations were barely significant, but the differences in body length of bugs reared on different varieties were highly significant.

TABLE 4.—*Average body length of chinch bugs reared on different varieties of sorghum in laboratory studies*

Variety	Average body length of adults			
	First generation	Second generation	Third generation	Average
	Millimeters	*Millimeters*	*Millimeters*	*Millimeters*
Wheatland	3.70	3.78	3.85	3.78
Dwarf Yellow milo	3.80	3.76	3.61	3.72
Honey sorgo	3.72	3.74	3.41	3.62
Sharon kafir	3.72	3.71	3.40	3.61
Blackhull kafir	3.61	3.54	3.55	3.57
Kansas Orange sorgo × Dwarf Yellow milo	3.48	3.49	3.50	3.49
Atlas sorgo	3.60	3.40	3.36	3.45
Feterita	3.34	3.57	3.07	3.35
Kansas Orange sorgo	3.06	3.35	(1)	3.20
Average	3.56	3.59	3.47	3.54
Differences required for significance at odds of 19 to 1				.21

[1] All dead before adult stage reached. Average of first and second generations used for analysis of variance.

EFFECT OF VARIETY ON RATE OF NYMPHAL DEVELOPMENT

A total of 307 chinch bugs was reared from egg to adult on 9 varieties of sorghum. Bugs were brought into the laboratory from the field and placed on the same varieties as those from which they had been collected. The eggs laid by females feeding on each variety were kept separate, and the resulting nymphs were placed on the same variety as that upon which the female had been feeding when the eggs were laid.

The records for nymphal development are summarized in table 5. The nymphal period ranged from 18 to 67 days, depending on the

TABLE 5.—*Days required for development of 3 generations of chinch bug nymphs reared in the laboratory on 9 varieties of sorghum*

Variety	Days from hatching to adult			
	First generation	Second generation	Third generation	Average
Dwarf Yellow milo	37.4	27.0	30.1	31.50
Wheatland	41.0	25.2	28.4	31.53
Honey sorgo	43.3	27.0	29.5	33.27
Blackhull kafir	39.4	27.9	34.4	33.90
Kansas Orange sorgo × Dwarf Yellow milo	39.1	27.2	43.9	36.73
Kansas Orange sorgo	43.0	31.1		37.05
Atlas sorgo	42.8	32.8	38.0	37.87
Sharon kafir	41.5	28.2	44.0	37.90
Feterita	46.5	29.3	49.0	41.60
Average	41.6	28.4	37.2	35.7

variety of sorghum and the temperature. In most cases nymphs feeding on those varieties that are most susceptible under field conditions matured faster than nymphs feeding on resistant varieties. On feterita, however, which is a moderately susceptible variety under field conditions, development was slower than on any of the resistant varieties. The fastest development recorded for any of the first-generation nymphs was 31 days for one nymph on Honey sorgo. The longest time required for nymphal development was 51 days,

as recorded for three nymphs, one each on Sharon kafir, feterita, and Honey sorgo.

The development of second-generation nymphs was slowest on Atlas sorgo. Second-generation nymphs developed faster on all varieties than those of the first generation because of the higher temperature, the period from egg to adult ranging from 20 to 42 days.

Third-generation nymphs showed the widest variation in rate of development. One nymph feeding on Wheatland developed from egg to adult in 18 days, and two nymphs, one on Sharon kafir and one on Kansas Orange sorgo × Dwarf Yellow milo, required 67 days to complete their development. There was a difference of 20.6 days in the average time required for nymphs to mature on Wheatland and feterita. This extreme variability was due to the warm weather prevailing during the first 28 days of this experiment, followed by a sudden change to cool weather, which lasted throughout the fall. Thus, the nymphal period of those bugs that had not reached the adult stage by the end of the twenty-eighth day of the experiment was greatly lengthened.

There was some variation in the length of stadia of nymphs feeding on each of the different varieties, but as a general rule this difference was not great, and in all cases the first stadium was the longest. Little difference existed between the second, third, and fourth stadia, but the fifth stadium was longer.

When the data were analyzed it was found that the differences between generations in rate of development were highly significant. Considering all the data, the differences in rate of development on the different varieties were not significant. Nevertheless, when the three most susceptible varieties were compared with the three most resistant varieties by Student's method, the odds were about 255 to 1 against a difference as great as this being due to chance alone.

EFFECT OF VARIETY ON MORTALITY OF NYMPHS

The nymphs were reared in mass culture until they were 5 days old, when they were put into individual cages. The mortality of nymphs was very high the first few days after hatching, but no records were kept while they were in mass culture, because it was difficult to distinguish between mortality due to handling and that due to variety of host.

The mortality of nymphs for all three generations is given in table 6. The lowest for first-generation nymphs was on Dwarf Yellow milo and the highest on Kansas Orange sorgo. Blackhull kafir produced the lowest mortality in the second generation, and Atlas sorgo the highest. The relative mortality of third-generation nymphs was similar to that of the first generation. However, the mortality of first instars was much greater than in the first and second generations.

The average nymphal mortality for all three generations was lowest for nymphs reared on Dwarf Yellow milo. Then in order came Wheatland, Blackhull kafir, Kansas Orange sorgo × Dwarf Yellow milo, Honey sorgo, Sharon kafir, feterita, Atlas sorgo, and Kansas Orange sorgo. From the analysis of variance it was found that the differences in the average mortality between generations and between varieties were both highly significant.

TABLE 6.—*Percent mortality of 3 generations of chinch bug nymphs reared in the laboratory on 9 varieties of sorghum*

Variety	Percent mortality of—			
	First generation	Second generation	Third generation	Average
Kansas Orange sorgo	92	65	100	85.7
Atlas sorgo	60	80	96	78.7
Feterita	56	65	80	67.0
Sharon kafir	48	40	76	54.7
Honey sorgo	36	45	52	44.3
Kansas Orange sorgo × Dwarf Yellow milo	24	45	40	36.3
Blackhull kafir	20	30	52	34.0
Wheatland	12	35	48	31.7
Dwarf Yellow milo	12	45	28	28.3
Average	40.0	50.0	63.6	51.2
Difference required for significance at odds of 19 to 1				8.9

HOST PREFERENCE

Laboratory tests were conducted in 1936 to study the preference of the chinch bug for different varieties of sorghum. A wooden cage 22 inches long, 6 inches wide, and 6 inches high, equipped with a sliding glass top, was constructed. In each end of the cage were large holes covered with fine-mesh copper screen, for ventilation. The cage was supported on 4 metal legs 10 inches long which were clamped on each corner. In the bottom of the cage 10 holes were bored 2 inches apart. One-ounce medicine bottles with screw tops were inserted in these holes from underneath the cage and filled with water. Seedling plants of two varieties to be compared were placed alternately in these bottles, so that the roots were submerged in the water and the upper portions extended into the cage. Cotton was packed around each plant at the mouth of the bottle to prevent escape of the insects. Adult chinch bugs were then collected from the field and placed in the cage. At intervals of about 2 hours the insects on each plant were counted and then removed and distributed evenly in the cage.

Atlas sorgo was compared successively with the eight other varieties in these tests. Table 7 shows the number and percentage of bugs attracted to each variety in five trials. These insects showed a distinct preference for Dwarf Yellow milo, Wheatland, and Honey sorgo, over Atlas sorgo, but only a very slight preference for the other varieties over Atlas sorgo.

HOST TOLERANCE

To study the tolerance of different varieties of sorghum to a uniform infestation of chinch bugs, single plants grown in 6-inch flowerpots (fig. 1), three of each variety, were brought into the laboratory when about 10 inches high and covered with 5- by 8-inch celluloid tubes. One hundred adult chinch bugs were placed on each plant.

Tolerance studies were made with eight varieties of sorghum (table 8). An average of only 88.3 hours was required for the bugs to kill Honey sorgo and Dwarf Yellow milo plants as compared with 131.7 hours for Atlas sorgo. Two plants of Atlas sorgo lived 135 hours, whereas all the plants of Honey sorgo and Dwarf Yellow milo were dead at the end of 95 hours. When these data were tested by analysis of variance, the differences between varieties were found to be highly significant.

790476—48——2

TABLE 7.—*Preference of chinch bugs for different varieties of sorghum as compared with Atlas sorgo, in laboratory tests*

Variety	Bugs feeding on plants							Bugs on each variety
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Total	Average per trial	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
Dwarf Yellow milo	115	95	96	34	43	383	76.6	64.26
Atlas sorgo	68	44	36	31	34	213	42.6	35.74
Blackhull kafir	91	92	84	42	40	349	69.8	52.32
Atlas sorgo	79	94	82	41	22	318	63.6	47.68
Honey sorgo	123	100	87	61	50	421	84.2	62.83
Atlas sorgo	68	66	50	36	29	249	49.8	37.17
Kansas Orange sorgo	42	61	149	46	45	343	68.6	49.86
Atlas sorgo	50	61	159	40	35	345	69.0	50.14
Wheatland	140	141	129	109	98	617	123.4	60.14
Atlas sorgo	116	103	77	52	61	409	81.8	39.86
Kansas Orange sorgo×Dwarf Yellow milo	118	85	71	75	74	423	84.6	53.61
Atlas sorgo	117	72	56	58	63	366	73.2	46.39
Sharon kafir	119	120	114	108	140	601	120.2	50.42
Atlas sorgo	133	103	108	99	148	591	118.2	49.58
Feterita	66	130	140	74	68	478	95.6	50.47
Atlas sorgo	60	150	135	59	65	469	93.8	49.53

FIGURE 1.—Cages used for tolerance studies with the chinch bug.

TABLE 8.—*Tolerance of 8 varieties of sorghum to a uniform infestation of chinch bugs under controlled conditions in the laboratory*

Variety	Condition of plant after indicated number of hours [1]											Average hours of life of plant
	35	45	55	65	75	85	95	105	115	125	135	
Honey sorgo	A	B	C	E	E	F						88.3
	A	A	B	C	C	E	F					
	A	A	B	D	E	F						
Dwarf Yellow milo	A	A	B	B	B	E	F					88.3
	A	B	B	B	C	F						
	A	A	B	C	D	F						
Wheatland	A	A	B	C	C	D	F					98.3
	A	A	A	B	C	C	E	F				
	A	A	B	C	D	E	F					
Kansas Orange × Dwarf Yellow milo	A	A	A	B	C	D	E	F				101.7
	A	A	B	B	D	E	F					
	A	A	A	B	C	C	E	F				
Blackhull kafir	A	B	B	C	C	D	E	E	F			108.3
	A	A	A	B	C	D	E	E	F			
	A	B	C	C	D	E	F					
Feterita	A	B	B	C	B	C	D	D	E	F		121.7
	A	A	A	B	C	D	D	D	E	F		
	A	B	B	B	C	D	E	E	F			
Kansas Orange sorgo	A	A	A	A	B	D	D	E	E	F		128.3
	A	A	A	A	B	D	D	E	E	F		
	A	A	A	A	B	C	D	D	E	E	F	
Atlas sorgo	A	A	A	A	B	B	C	C	D	F		131.7
	A	A	A	A	B	B	B	B	C	E	F	
	A	A	A	A	B	B	C	C	D	E	F	

[1] A, no injury; B, basal leaves slightly wilted; C, part of basal leaves permanently wilted; D, about half of the leaves of the plant permanently wilted; E, entire plant nearly dead; F, entire plant dead.

FIELD STUDIES

CONDITIONS AND METHODS

In 1937 the effect of different varieties of older sorghum plants on the biology of the chinch bug was studied under field conditions on Dwarf Yellow milo, feterita, Blackhull kafir, and Atlas sorgo. Three series of each variety were used—(1) plants 20 to 30 days old (average 25 days), (2) plants 40 to 50 days old (average 45 days), and (3) plants at the heading stage.

The bugs were confined in cages on plants growing in the field. These cages consisted of celluloid tubes about 7 by 2.5 cm. (fig. 2), with a cork stopper covered with muslin in each end. An ellipsoid hole about the middle of the side of the tube was also covered with muslin, which was attached with waterproof glue. Each cage was fastened to a sorghum stalk with a fabric-covered elastic band so that the hole was next to the stalk. Bugs placed in these cages appeared to have no difficulty in feeding through the cloth. The cages were placed on the north side of the plants and as much in the shade as possible. Under these conditions the temperature in the cages ordinarily ranged from 2° to 6° F. higher than outside shade-air temperatures. The shade-air temperatures during these experiments are shown in figure 3.

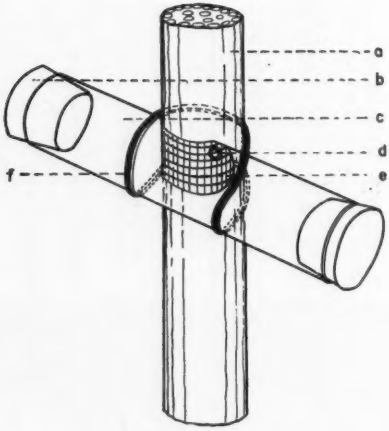

FIGURE 2.—Cage used to confine chinch bugs on plants growing in the field: *a*, Sorghum plant; *b*, cork stopper; *c*, celluloid cage; *d*, chinch bug; *e*, feeding area; *f*, elastic band.

The bugs used for oviposition and longevity studies were collected in the field while in the fifth instar. The first-generation bugs were

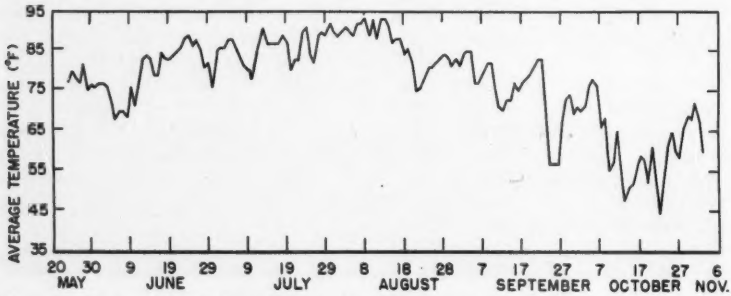

FIGURE 3.—Average daily shade-air temperatures in sorghum fields at Lawton, Okla., May to November 1937.

collected from spring barley and the second-generation from the same varieties of sorghum of which they were to be confined in the cages. These fifth instars were then placed on plants of the same variety and age as those on which they were later to be tested. When the bugs reached the adult stage, one pair was placed in each cage and one cage attached to each plant. The nymphs used for studying the rate of development were from eggs laid by bugs that had fed on plants of the same variety and age as those upon which they were later to be tested.

EFFECT OF VARIETY AND AGE OF PLANT ON OVIPOSITION

The oviposition records of chinch bugs on four varieties of sorghum at three stages of growth are given in table 9.

The bugs feeding on Dwarf Yellow milo laid the most eggs, but the differences in oviposition on varieties were not so great as when similar tests were made with seedling plants in the laboratory. Bugs feeding on Dwarf Yellow milo laid nearly twice as many eggs as those feeding on Atlas sorgo or Blackhull kafir. Feterita was intermediate between Dwarf Yellow milo and Blackhull kafir in this respect.

TABLE 9.—*Number of eggs per female of 2 generations of chinch bugs on 4 varieties of sorghum at 3 different ages growing in the field*

Variety	Generation of bugs	Eggs laid per female on—			
		25-day-old plants	45-day-old plants	Headed plants	Average of all plants
		Number	*Number*	*Number*	*Number*
Dwarf Yellow milo	First	123.9	201.5	203.1	
	Second	113.4	219.9	270.8	188.8
Feterita	First	88.4	135.8	167.4	
	Second	86.2	230.5	183.9	148.7
Blackhull kafir	First	61.9	145.3	107.7	
	Second	72.9	116.5	123.6	104.6
Atlas sorgo	First	78.9	98.0	101.2	
	Second	32.8	158.8	118.0	97.9
Average		82.3	163.3	159.5	135.0

Fewer eggs were laid on 25-day-old plants of all varieties than on 45-day-old or headed plants. Bugs feeding on Dwarf Yellow milo laid more eggs on headed plants than on plants of either of the other two stages, whereas bugs feeding on feterita, Blackhull kafir, and Atlas sorgo laid the most eggs on 45-day-old plants. The analysis of variance showed that the differences in numbers of eggs laid on different varieties and on plants of different ages are highly significant, but that the difference between generations is not significant.

EFFECT OF VARIETY AND AGE OF PLANT ON LONGEVITY

Longevity records of the females used in the oviposition experiment (table 10) showed no correlation between the length of life and the number of eggs laid (table 9). Bugs feeding on feterita lived on an average 10 days longer than bugs feeding on the other varieties.

TABLE 10.—*Longevity of 2 generations of chinch bugs on 4 varieties of sorghum at 3 different ages in the field*

Variety	Generation of bugs	Longevity of bugs on—			
		25-day-old plants	45-day-old plants	Headed plants	Average of all plants
		Days	*Days*	*Days*	*Days*
Dwarf Yellow milo	First	31.6	47.2	43.6	
	Second	36.2	46.7	54.2	43.2
Feterita	First	43.2	46.3	53.3	
	Second	41.1	80.2	56.7	53.5
Blackhull kafir	First	29.0	53.7	33.1	
	Second	41.8	48.6	55.5	43.6
Atlas sorgo	First	41.5	43.6	42.5	
	Second	22.1	54.4	50.2	42.4
Average		35.8	52.6	48.6	45.7

The age of the plant, however, had a similar effect on the longevity as on oviposition of the bugs, for they lived a shorter time on 25-day-old plants than on plants of either of the other two age groups. Bugs feeding on Dwarf Yellow milo lived longest on headed plants, while those feeding on feterita, Blackhull kafir, and Atlas sorgo plants lived longest on 45-day-old plants. When these data were tested by analysis of variance, the differences in longevity among varieties and between generations were not found to be significant, but the differences between the three ages of plants were highly significant.

EFFECT OF VARIETY AND AGE OF PLANT ON SIZE OF ADULTS

The average body lengths of three generations of chinch bugs reared on the four varieties are shown in table 11. Bugs reared on

TABLE 11.—*Average body length of adult chinch bugs reared in the field on 4 varieties of sorghum at 3 different ages*

Variety	Average body length of adults on—			
	25-day-old plants	45-day-old plants	Headed plants	Average of all plants
	Millimeters	*Millimeters*	*Millimeters*	*Millimeters*
Dwarf Yellow milo	3.84	3.80	3.68	3.77
Feterita	3.68	3.80	3.63	3.70
Blackhull kafir	3.63	3.76	3.76	3.72
Atlas sorgo	3.67	3.60	3.42	3.5

Dwarf Yellow milo were, on an average, distinctly larger than bugs reared on Atlas sorgo but only slightly larger than those reared on feterita or Blackhull kafir. In the analysis of variance, none of these differences were found to be significant. The age of the plants did not significantly affect the size of the bugs.

EFFECT OF VARIETY AND AGE OF PLANT ON RATE OF NYMPHAL DEVELOPMENT

When nymphs were reared from egg to adult on four varieties of sorghum under field conditions, they developed most rapidly on Dwarf Yellow milo (table 12). The rate of development on feterita, Black-

TABLE 12.—*Days required for development of 3 generations of chinch bug nymphs in the field on 4 varieties of sorghum at 3 different ages*

Variety	Generation of bugs	Time required for development—			
		25-day-old plants	45-day-old plants	Headed plants	Average of all plants
		Days	*Days*	*Days*	*Days*
Dwarf Yellow milo	First	35.7	32.5	30.5	36.1
	Second	22.9	24.5	24.6	
	Third	46.7	50.5	56.6	
Feterita	First	34.3	35.0	36.5	40.4
	Second	26.7	27.5	27.0	
	Third	60.3	58.9	57.3	
Blackhull kafir	First	33.5	39.0	43.8	41.3
	Second	28.2	25.6	27.0	
	Third	59.3	54.9	60.7	
Atlas sorgo	First	39.0	38.0	38.0	39.4
	Second	26.8	25.5	27.0	
	Third	54.2	51.3	55.0	
Average		39.0	38.6	40.3	39.3

hull kafir, and Atlas sorgo was about the same. Second-generation bugs developed much faster than first- and third-generation bugs, owing to higher prevailing temperatures. Analysis of variance showed that the differences in the rate of development on the different varieties and in different generations were highly significant, but on the plants of different ages they were not significant.

EFFECT OF VARIETY AND AGE OF PLANT ON MORTALITY OF NYMPHS

The mortality of nymphs feeding on the four varieties is shown in table 13. The average mortality for each generation was lowest on Dwarf Yellow milo, and in all except 25-day-old plants the mortality was highest on Atlas sorgo. Nymphs feeding on Blackhull kafir had a higher mortality than those feeding on feterita. Analysis of variance showed that the differences in mortality between varieties were significant. The average mortality on all four varieties was slightly higher on headed plants than on the younger plants, but the difference was not significant. The differences in mean mortalities between the generations were significant.

TABLE 13.—*Mortality of 3 generations of chinch bug nymphs on 4 varieties of sorghum at 3 different ages growing in the field*

Variety	Generation of bugs	Mortality of nymphs on—			
		25-day-old plants	45-day-old plants	Headed plants	Average of all plants
		Percent	*Percent*	*Percent*	*Percent*
Dwarf Yellow milo	First	72.0	84.0	84.0	62.7
	Second	68.0	60.0	52.0	
	Third	36.0	48.0	60.0	
Feterita	First	88.0	96.0	92.0	74.7
	Second	76.0	68.0	64.0	
	Third	76.0	36.0	76.0	
Blackhull kafir	First	92.0	88.0	100.0	77.8
	Second	80.0	68.0	68.0	
	Third	68.0	64.0	72.0	
Atlas sorgo	First	76.0	96.0	96.0	83.1
	Second	80.0	84.0	84.0	
	Third	80.0	76.0	76.0	
Average		74.3	72.3	68.7	71.8

DISCUSSION

Little is known as to the basis of chinch bug resistance in plants, but it is believed to be related to the condition or composition of the cell sap rather than to any readily observable morphological characters of the plant. The laboratory data on the length of life, fecundity, rate of development, and size of the insect indicate that seedlings of different sorghum varieties do not serve equally well as food. Generally chinch bugs feeding on those varieties that are susceptible under field conditions live longer and lay more eggs than those feeding on resistant varieties. The nymphs also develop into larger adults. This is not true in all cases, however, feterita being the outstanding exception. Kansas Orange sorgo X Dwarf Yellow milo showed transgressive segregation according to field results but not in the laboratory tests.

Whether fecundity, mortality, and size are all influenced by one genetic factor, each by a separate factor, or each by several factors, is not known. The fact that a low fecundity was accompanied by a high death rate would indicate that all may be affected by the same factor, or by a group of factors that are closely linked. Whether these differences are due to absence, presence, or to the quantity of certain food materials in the cell sap is not known.

The differences between chinch bugs reared on different varieties of sorghum were not so marked for older plants under field conditions as for seedling plants tested in the laboratory. In fact, under field conditions the only significant differences due to variety were in number of eggs laid, nymphal mortality, and rate of nymphal development. The apparent discrepancy respecting the relative position of resistance of feterita has been cleared up to some extent by field-cage studies. In the laboratory this variety, which is moderately susceptible under field conditions, has always reacted as resistant when the effect of host on the biology of the chinch bug was used as a criterion. In studies with older plants bugs laid more eggs and the mortality of nymphs was lower on this variety than on either Blackhull kafir or Atlas sorgo. The reactions of the bugs as determined by both field-cage tests and field observations indicate it to be more susceptible than Blackhull kafir or Atlas sorgo.

SUMMARY

Varieties of sorghum differ greatly in their ability to withstand the attack of the chinch bug (*Blissus leucopterus* (Say)). In general, under field conditions the milos are very susceptible, the feteritas susceptible, and the kafirs and sorgos rather resistant.

A study of the effect of seedling plants of nine varieties of sorghum on the biology of the chinch bug showed that chinch bug females feeding on Dwarf Yellow milo laid more eggs than those feeding on any of the other varieties. In the order of their increasingly detrimental effect on chinch bug fecundity, came Wheatland, Honey sorgo, Blackhull kafir, Sharon kafir, Kansas Orange sorgo × Dwarf Yellow milo, Atlas sorgo, feterita, and Kansas Orange sorgo.

There was a marked difference in the longevity of females when confined with different varieties of sorghum, but longevity was not

always correlated with the total number of eggs laid. Chinch bug females lived longest on Wheatland, and the shortest time on Kansas Orange sorgo.

Nymphs reared on the susceptible varieties were larger than those reared on the resistant varieties. There were some differences in the rate of development of nymphs on the several varieties. While these differences among all varieties were not significant when tested by analysis of variance, comparison by Student's method of results for the three most resistant and the three most susceptible varieties showed that nymphs develop significantly faster on the susceptible varieties. Mortality of nymphs was greatest on Kansas Orange sorgo and least on Dwarf Yellow milo.

Under laboratory conditions adult chinch bugs showed a preference for susceptible varieties over resistant varieties. The resistant varieties showed the greatest tolerance to a uniform infestation of adult chinch bugs.

The effect of four varieties of sorghum at three stages of growth on the biology of the chinch bug was also studied under field conditions. The bugs laid the largest number of eggs on Dwarf Yellow milo, the next largest on feterita, and the smallest number on Blackhull kafir and Atlas sorgo. Fewer eggs were laid on all varieties of 25-day-old plants than on plants 45 days old or on plants in the heading stage. Chinch bugs lived longer on 45-day-old and headed plants than on 25-day-old plants. There was no apparent correlation between resistance of plants and longevity of adult chinch bugs.

When chinch bugs were reared in field cages, the adults were distinctly larger on Dwarf Yellow milo than on Atlas sorgo, those reared on feterita or Blackhull kafir being intermediate in size. Generally, bugs reared on headed plants were smaller than those reared on younger plants. Under field conditions nymphs developed faster on Dwarf Yellow milo than on the other three varieties. The age of plant did not affect the rate of development of nymphs. Nymph mortality was lowest on Dwarf Yellow milo, highest on Atlas sorgo, and intermediate on feterita and Blackhull kafir.

It is believed that the condition or composition of the cell sap rather than any readily observable morphological characters of the plant is responsible for the differences observed on the biology of the chinch bug when feeding on different varieties and ages of sorghum. Whether these differences are due to absence, presence, or quantities of certain food materials in the cell sap is not known.

LITERATURE CITED

(1) Blanchard, R. A., and Dudley, J. E., Jr.
1934. Alfalfa plants resistant to the pea aphid. Jour. Econ. Ent 27: 262–264.

(2) Dahms, R. G., Snelling, R. O., and Fenton, F. A.
1936. Effect of several varieties of sorghum and other host plants on biology of the chinch bug. Jour. Econ. Ent. 29: 1147–1153, illus.

(3) DeLong, D. M., and Jones, M. P.
1926. Control measures for the Houghton gooseberry aphis with special reference to plant resistance. Jour. Econ. Ent. 19: 40–43, illus.

790476—48——3

(4) Hodge, Charles, IV.
1933. growth and nutrition of melanoplus differentialis thomas (orthoptera: acrididae) i. growth on a satisfactory mixed diet and on diets of single food plants. Physiol. Zool. 6: 306–328, illus.
(5) Isely, D.
1935. relation of hosts to abundance of cotton bollworm. Ark. Agr. Expt. Sta. Bul. 320, 30 pp., illus.
(6) McColloch, J. W., and Salmon, S. C.
1918. relation of kinds and varieties of grain to hessian-fly injury. Jour. Agr. Res. 12: 519–527.
(7) Oklahoma Agricultural Experiment Station.
1936. Chinch-bug control studied from several angles. *In* Science Serving Agriculture. Okla. Agr. Expt. Sta. [Bien.] Rpt. 1934–36, pp. 34–39, illus.
(8) Painter, R. H.
1936. the food of insects and its relation to resistance of plants to insect attack. Amer. Nat. 70: 547–566.
(9) Seamans, H. L., and McMillan, E. L.
1935. the effect of food plants on the development of the pale western cutworm (agrotis orthogonia morr.). Jour. Econ. Ent. 28: 421–425.
(10) Snelling, R. O., Painter, R. H., Parker, J. H., and Osborn, W. M.
1937. resistance of sorghums to the chinch bug. U. S. Dept. Agr. Tech. Bul. 585: 56 pp., illus.
(11) Snelling, R. O., and Dahms, R. G.
1937. resistant varieties of sorghum and corn in relation to chinch bug control in oklahoma. Okla. Agr. Expt. Sta. Bul. 232, 22 pp., illus.
(12) Vinall, H. N., Stephens, J. C., and Martin, J. H.
1936. identification, history, and distribution of common sorghum varieties. U. S. Dept. Agr. Tech. Bul. 506, 102 pp., illus.
(13) Wadley, F. M.
1931. ecology of toxoptera graminum, especially as to factors affecting importance in the northern united states. Ent. Soc. Amer. Ann. 24: 325–395, illus.
(14) Winter, J. D.
1929. a preliminary account of the raspberry aphids. Minn. Agr. Expt. Sta. Tech. Bul. 61, 30 pp., illus.

EFFECT OF NITROGEN, PHOSPHORUS, AND POTASSIUM ON SUSCEPTIBILITY OF TOMATOES TO ALTERNARIA SOLANI [1]

By H. Rex Thomas

Associate pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

An important disease of tomatoes (*Lycopersicon esculentum* Mill.) is caused by *Alternaria solani* (Ell. and G. Martin) L. R. Jones and Grout. This disease, which attacks the stem, leaf, and fruit of tomato, is particularly serious on seedlings grown in the Southern States for setting the northern canning acreage. In such districts in the North this fungus often causes a severe leaf blight and frequently an appreciable amount of stem-end infection of the fruit. It has been commonly observed that the disease appears to be worse on plants located in the less fertile parts of a field, on earlier transplanted plants, and on earlier maturing varieties. Moore and Thomas (*3*) [2] found that the infection of tomato seedlings by *A. solani* increases with age. Horsfall and Heuberger (*2*) and Barratt and Richards (*1*) reported that a direct relation exists between yield and defoliation. Thomas (*5*) found that young tomato seedlings grown in the greenhouse in sand with a high nitrogen supply were consistently less susceptible to the collar rot type of stem canker caused by *A. solani* and had smaller lesions than those raised in medium- or low-nitrogen solutions. Horsfall and Heuberger (*2*) found less infection on field-grown tomatoes that received sodium nitrate. They believed that the reduction was due to an overvegetative condition of the plant accompanied by a poor fruit set. Barratt and Richards (*1*) reported that the results of culture tests of various levels of nitrogen in greenhouse sand after artificial inoculation were inconclusive.

The present paper presents the data obtained in 1941 and 1942 from three greenhouse experiments and one field experiment in which the influence of different levels of nitrogen (N), phosphorus (P), and potassium (K) nutrition and their combination on the susceptibility of tomatoes to infection by *Alternaria solani* was studied.

[1] Received for publication April 3, 1947. Cooperative investigations of the Division of Fruit and Vegetable Crops and Diseases and the Department of Botany and Plant Pathology, Purdue University Agricultural Experiment Station. Conducted as part of the southern plant-bed project in which the Georgia Coastal Plain Experiment Station, the Georgia Agricultural Experiment Station, the Georgia Department of Entomology, and the New Jersey Agricultural Experiment Station also participated. Journal Paper No. 288, Purdue University Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 306.

MATERIALS AND METHODS

GREENHOUSE EXPERIMENTS

The variety Indiana Baltimore was used in all the experiments. The greenhouse tests were made at La Fayette, Ind., during fall and spring when the amount of light was sufficient to produce suitable growth.

The plants were grown in quartz sand, 1 part flint grade to 5 parts diamond grade, [3] and were supplied with a complete nutrient solution until they were approximately 8 inches tall. They were then transplanted to fresh sand in 6-inch white glazed pots in the first series and to 2-gallon white glazed crocks in the last two; different nutrient solutions were supplied to the plants. This procedure was followed in order to obtain plants of approximately the same size, but deficient in different amounts of the respective nutrient elements. The plants were transplanted to clean sand and containers before they were given the different treatments, because of the difficulty of removing all of the residues of nutrients applied during the preinoculation period. Four one-plant replicates on each nutrient solution were arranged in randomized blocks on the greenhouse bench.

The sand medium was flushed daily with the solution, the amount being increased with the age of the plant. Distilled water was used in the first series, but tap water was used in the other two. Additional water when needed was supplied during the day. Since the composition of the nutrient solution varied slightly in the different series, the formulas are presented in the respective descriptions. Changes in the composition of the solution were made when necessary to keep the plants at the desired condition of growth and level of nutrition; for example, a sharp reduction in the amount of sunshine could make a relatively low-N plant become either a medium- or a high-N plant and, conversely, a high-N plant might become low in N. Frequent tissue tests were made according to the methods described by Thornton, Conner, and Fraser (*7*) to determine qualitatively the available nitrogen, phosphorus, and potassium. An effort was made to regulate the low levels of nutrients so that there would be no severely stunted plants. When the plants on the complete preinoculation solution were approximately in the 11- to 12-leaf stage, they were inoculated with a culture of *Alternaria solani* that produced numerous conidia (*6*). The conidia were washed from 8-day-old petri-dish cultures and suspended in water. A De Vilbiss atomizer operated at about 15 pounds' pressure was used to apply the suspension. After inoculation the plants were kept moist for 48 hours at a temperature of 70° to 75° F. Incubation for longer periods resulted in such severe infection that differences were less apparent. One plant from each nutrient treatment sprayed with water alone served as a check.

When possible the temperature of the greenhouse was kept at approximately 75° F. during the day and at 70° at night. In each experiment there was added to each of the different solutions 2 millimoles of $MgSO_4.7H_2O$ (48.6 p. p. m. of Mg), 5 p. p. m. of Fe, 0.5 p. p. m. of Mn, 0.05 p. p. m. of Cu, 0.05 p. p. m. of Zn, and 0.5 p. p. m. of B. The pH was adjusted to approximately 6 by using HCl or NaOH.

[3] Obtained from Ottawa Silica Co., Ottawa, Ill.

Fruit was allowed to form in the first experiment, but not in the second or third.

Susceptibility of the plants was based on the size of the leaf spots and the number of dead leaves occurring within a certain period after inoculation. The largest diameter was used in estimating the size of a leaf spot. In the first and third experiments the estimated sizes were grouped into four classes: (1) Less than 1 mm.; (2) 1 to 1.9 mm.; (3) 2 to 2.9 mm.; and (4) 3 mm. and larger. In experiment 2 the first three classes were the same, but the fourth represented spots 3 to 3.9 mm. and a fifth those 4 mm. and larger.

Statistical calculations were made according to methods of Snedecor (*4*). Significant differences are indicated by odds of 19:1 (the 5-percent level of *t*). The averages presented in the tables were rounded off from the original data, and the same values will not necessarily be obtained by averaging these rounded-off values.

Experiment 1 was conducted from August 15 to December 17, 1941. Two sets of plants were grown in sand supplied with 12 different nutrient solutions (see table 2) comprising all combinations of 3 levels of N, 2 of P, and 2 of K. One set of 48 plants received only these nutrients throughout the study. A second set of plants was similarly treated until the time of inoculation; then the differential treatments were discontinued and a solution high in N, P, and K was supplied to all plants until the end of the experiment. The first set is described as receiving "continued" treatment and the second as receiving "changed" treatment.

During the preinoculation period the low-N plants received 28.0 p. p. m. of N from $Ca(NO_3)_2.4H_2O$, the medium-N 84.0, and the high-N 252.1. The low-P plants received no P, and the high-P ones received 93.1 p. p. m. from KH_2PO_4 or $Ca(H_2PO_4)_2.H_2O$ depending on whether the chemical was in combination with low or high K. The low-K plants were not supplied with K because of the reserve already present in them, and the high-K plants received 351.9 p. p. m. of K from KCl or KH_2PO_4 depending on the phosphate level of the treatment. Additional $CaCl_2$ was added to maintain the calcium level at 841.5 p. p. m.

When the inoculations were made on November 21, 1941, the medium-N plants were closer in appearance to the low-N plants than to the high-N ones. The low-K plants, although not testing low according to tissue tests, were much smaller than the high-K ones. The high-N, high-P, high-K plants tested low in available phosphate several times during the progress of the experiment, but no deficiency symptoms were observable in the leaves. This indicates that not enough P had been added to supply the plants on this treatment. The susceptibility of these particular plants might have been different if they had been high in P throughout the experiment. Ten days after inoculation the sizes of the leaf spots on all the leaves were determined, and 22 days after inoculation living and dead leaves per plant were counted. The significant difference listed for the changed plants was obtained by using the data for the entire experiment and that for the continued ones by using the continued data only. The latter figure was calculated in order to make possible a more accurate comparison of treatments within the continued part of the experiment.

Experiment 2 was conducted from January 21 to April 29, 1942. Two levels each of N, P, and K in all combinations were supplied to the plants, a total of eight different treatments. The composition of the nutrient solution in parts per million was low-N 84.0 and high-N 280.2 (added as $NaNO_3$); low-K 78.2 and high-K 312.8 (added as KCl); and low-P 7.8 and high-P 31.0 (added as $Ca(H_2PO_4)_2.H_2O$). Calcium chloride was added in a quantity necessary to maintain the calcium level at 400.8 p. p. m. Sodium chloride was added in a quantity necessary to maintain similar osmotic pressures among the treatments and to keep the sodium level at approximately 459.9 p. p. m. During the experiment the intended high-P plants became low in available P, according to plant-tissue tests. Apparently the amount of P added once daily was not sufficient to maintain an excess in the plant tissues during periods of rapid growth. From March 4 the sand cultures were flushed twice daily with their respective solutions. Inoculations were made March 9.

The average size of the leaf spots on the fifth leaf above the cotyledons 10 days after inoculation and the average percentage of leaves dead per plant 28 days after inoculation were determined (see table 3).

TABLE 1.—*Composition of nutrient solutions, experiment 3*

Treatment	Amount of 1-molar solution per liter [1]							
	$NaNO_3$	KNO_3	NaH_2PO_4	KCl	NaCl	$CaCl_2$	$MgSO_4$	$Ca(NO_3)_2$
	Milliliters	*Milliliters*	*Milliliters*	*Milliliters*	*Milliliters*	*Milliliters*	*Milliliters*	*Milliliters*
Low nitrogen:								
Low phosphorus and low potassium	1	1	0	0	3	5	2	0
Low phosphorus and medium potassium	0	2	0	1	3	5	2	0
Low phosphorus and high potassium	0	2	0	7	5	5	2	0
High phosphorus and low potassium	1	1	2	0	1	5	2	0
High phosphorus and medium potassium	0	2	2	1	3	5	2	0
High phosphorus and high potassium	0	2	2	7	3	5	2	0
Medium nitrogen:								
Low phosphorus and low potassium	7	1	0	0	0	5	2	0
Low phosphorus and medium potassium	5	3	0	0	0	5	2	0
Low phosphorus and high potassium	0	8	0	1	5	5	2	0
High phosphorus and low potassium	7	1	2	0	0	5	2	0
High phosphorus and medium potassium	7	1	2	2	0	5	2	0
High phosphorus and high potassium	0	8	2	1	3	5	2	0
High nitrogen:								
Low phosphorus and low potassium	9	1	0	0	0	0	4	11
Low phosphorus and medium potassium	7	3	0	0	0	0	4	11
Low phosphorus and high potassium	9	9	0	0	0	0	4	7
High phosphorus and low potassium	5	1	2	0	0	0	4	13
High phosphorus and medium potassium	9	3	2	0	0	0	4	10
High phosphorus and high potassium	7	9	2	0	0	0	4	8

[1] Micronutrients added and pH adjusted as described in text (p. 290). For the high-N plants in this experiment the amount of $MgSO_4$, however, was doubled.

In experiment 3, August 5 to December 12, 1942, the plants were grown in sand supplied with 18 different nutrient solutions, represent-

ing all combinations of 3 levels of N and K and 2 of P (table 1). On a parts per million basis the N levels were 28.0, 112.1, and 448.2; P 0 and 62.0; and K 39.1, 117.3, and 351.9.

Ten days after inoculation the sizes of the leaf spots on the sixth and ninth leaves above the cotyledons were determined. Thirteen days after inoculation three leaves having spots less than 1 mm. in diameter were tagged on each plant, and 21 days later the average size of these leaf spots was determined. The living and dead leaves per plant were counted 34 days after inoculation (see table 4).

FIELD EXPERIMENTS

In 1942 a study was made of the influence of different levels and combinations of N, P, and K fertilization of tomato plants on their susceptibility to *Alternaria solani* infection in the field. Part of a field with a Crosby silt loam of low fertility and another part of the same field with a Brookston silty clay loam of high fertility were selected for the test. All combinations of low and high levels of N, P, and K were used—a total of 8 treatments. There were 4 replicates on the Crosby and 4 on the Brookston soil. Each individual plot consisted of 20 plants, 4 plants wide and 5 plants long, set 3½ by 3½ feet. Records were taken on the 6 plants in the center of the plot.

The high levels of the fertilizers applied to the soil were N (120 pounds per acre as ammonium sulfate), P (400 pounds of P_2O_5 per acre as superphosphate), and K (200 pounds of K_2O per acre as muriate of potash). On the Crosby loam the low levels of N and P were 5 percent of the high levels. No N or P was added to the low-N and low-K plots on the Brookston loam. No K was added to the low-K plot on either soil type. The fertilizer was broadcast on top of the soil and plowed under April 23. The plants, Indiana Baltimore variety, were set May 26.

The plants were sprayed with a water suspension of conidia of *Alternaria solani* in mid-July and again in early August. Defoliation records were based upon the percentage of leaves dead on the 3 main stems of each of the 6 center plants in the plot. The average for the 18 stems was used as the percentage for the plot. The first defoliation counts were made August 13. The second counts were made September 2 on the Crosby plot only. At this time the plants on the Brookston plot were very badly defoliated and there were no observable differences among treatments (see table 5). Early yields per acre were determined August 29.

GREENHOUSE RESULTS

SIZE OF LEAF SPOTS

The smallest leaf spots in experiment 1 (continued group) were found on the medium-N, high-P, low-K plants (table 2); in experiment 2 on the low-N, low-P, low-K plants (table 3); and in experiment 3 (sixth leaf) on the low-N, high-P, low-K plants (table 4). The largest leaf spots in experiments 1 and 2 occurred on the high-N, low-P, high-K plants and in experiment 3 (sixth leaf) on the high-N, low-P, medium-K plants. Representative leaves of plants grown on the 18 solutions of experiment 3 are shown in figures 1 to 3.

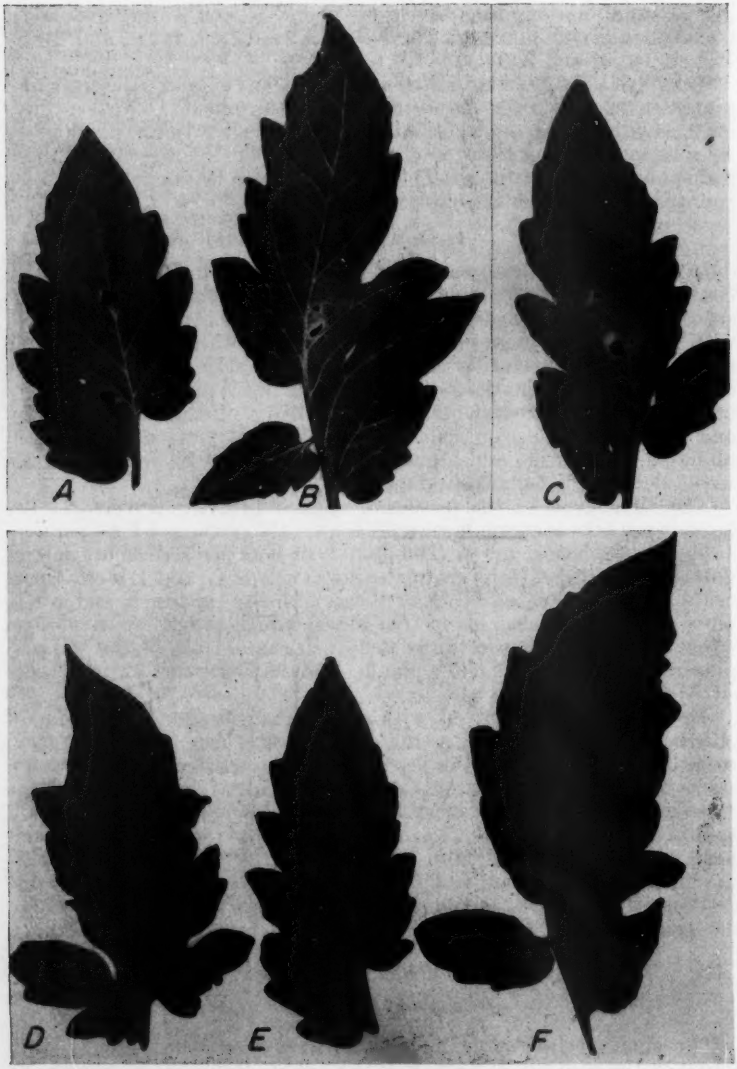

FIGURE 1.—Leaves (sixth above cotyledons) from tomato plants grown at a low-nitrogen level, showing susceptibility to *Alternaria solani*, experiment 3: *A*, Low phosphorus and low potassium; *B*, low phosphorus and medium potassium; *C*, low phosphorus and high potassium; *D*, high phosphorus and low potassium; *E*, high phosphorus and medium potassium; *F*, high phosphorus and high potassium.

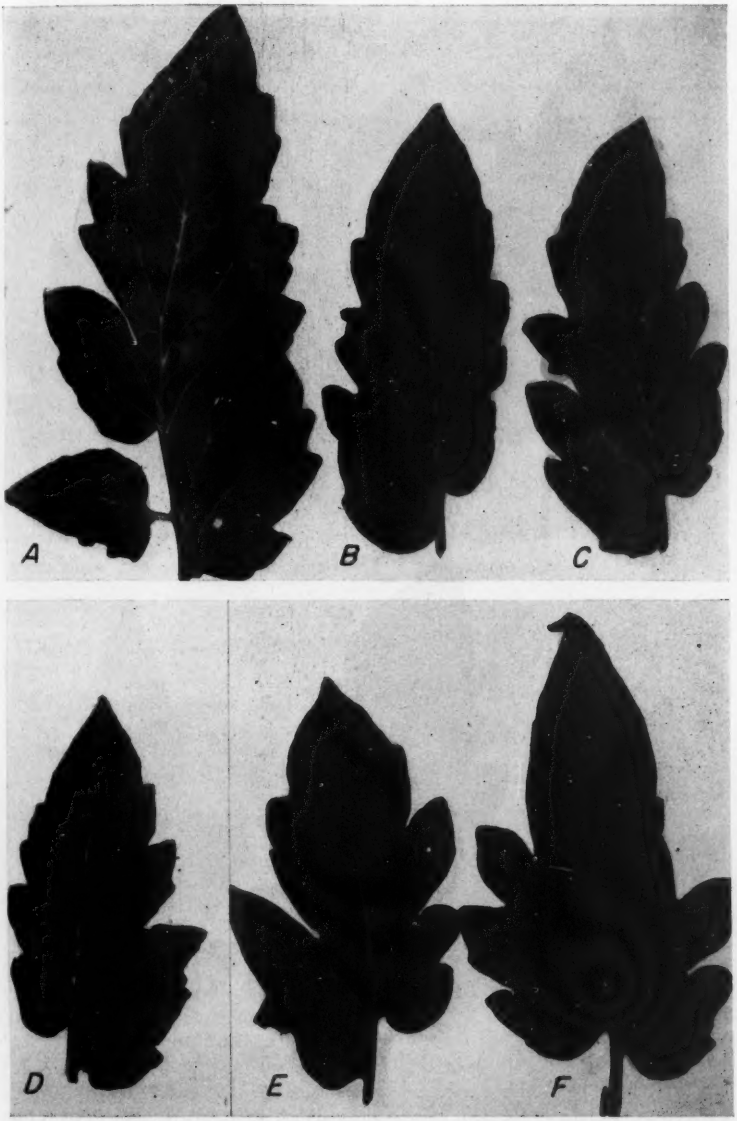

FIGURE 2.—Leaves (sixth above cotyledons) from tomato plants grown at a medium-nitrogen level, showing susceptibility to *Alternaria solani*, experiment 3: *A*, Low phosphorus and low potassium; *B*, low phosphorus and medium potassium; *C*, low phosphorus and high potassium; *D*, high phosphorus and low potassium; *E*, high phosphorus and medium potassium; *F*, high phosphorus and high potassium.

790476—48——4

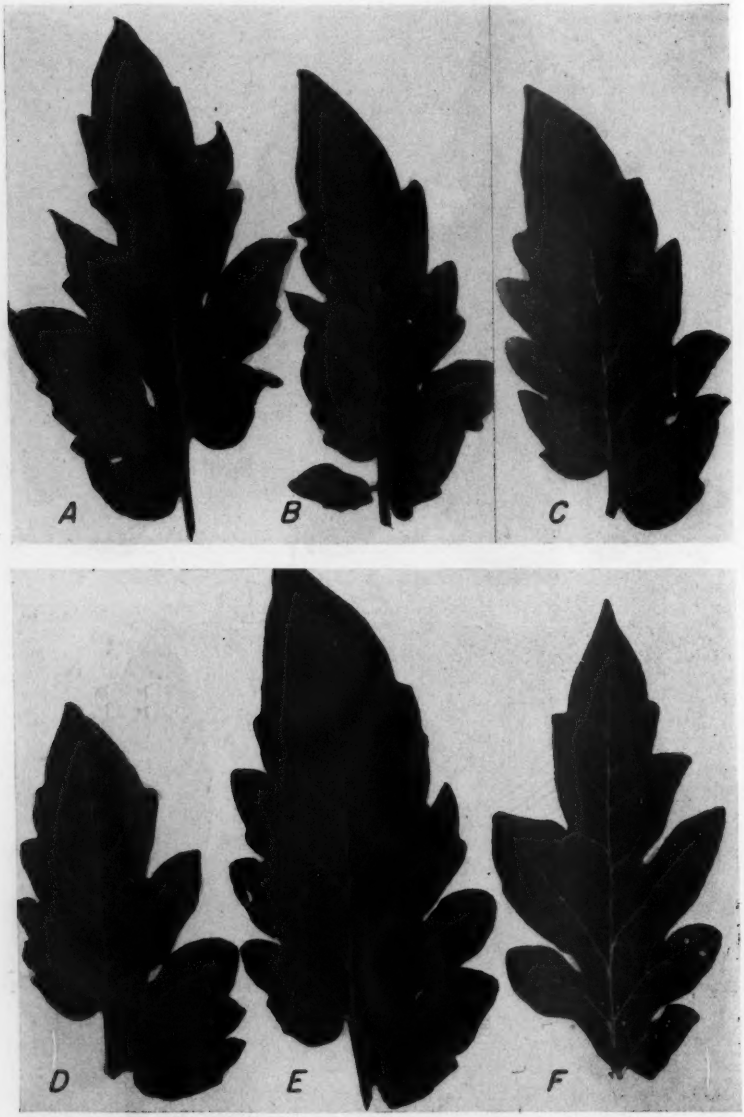

FIGURE 3.—Leaves (sixth above cotyledons) from tomato plants grown at a high-nitrogen level, showing susceptibility to *Alternaria solani*, experiment 3: *A*, Low phosphorus and low potassium; *B*, low phosphorus and medium potassium; *C*, low phosphorus and high potassium; *D*, high phosphorus and low potassium; *E*, high phosphorus and medium potassium; *F*, high phosphorus and high potassium.

TABLE 2.—*Susceptibility of tomato leaves to Alternaria solani as influenced by different levels and combinations of nitrogen, phosphorus, and potassium nutrition before and after inoculation, experiment 1*

[Each average based on 4 plants; 10 days after inoculation for size of leaf spots and 22 days after inoculation for dead leaves]

Nutrition treatment before inoculation	Average class [1] for size of leaf spot, with indicated treatment after inoculation		Leaves dead, with indicated treatment after inoculation	
	Continued	Changed [2]	Continued [2]	Changed [2]
			Percent	*Percent*
Low nitrogen:				
Low phosphorus and low potassium	1.1	1.2	14	15
Low phosphorus and high potassium	1.2	1.0	29	34
High phosphorus and low potassium	1.2	1.0	17	10
High phosphorus and high potassium	1.2	1.1	38	27
Medium nitrogen:				
Low phosphorus and low potassium	1.3	1.1	30	11
Low phosphorus and high potassium	1.2	1.1	37	25
High phosphorus and low potassium	1.0	.8	17	9
High phosphorus and high potassium	1.1	1.2	26	19
High nitrogen:				
Low phosphorus and low potassium	1.2	1.2	42	10
Low phosphorus and high potassium	1.6	1.1	53	30
High phosphorus and low potassium	1.1	1.1	13	12
High phosphorus and high potassium	1.3	1.1	10	14
Difference required for significance (5-percent level)	.2	.2	16	13
Nitrogen averages:				
Low	1.2	1.1	25	21
Medium	1.1	1.0	28	16
High	1.3	1.1	30	17
Difference required for significance (5-percent level)	.1	.1	8	6
Phosphorus averages:				
Low	1.3	1.1	34	21
High	1.2	1.1	21	15
Difference required for significance (5-percent level)	.1	.1	7	4
Potassium averages:				
Low	1.2	1.1	22	11
High	1.3	1.1	32	25
Difference required for significance (5-percent level)	.1	.1	7	4
Treatment after inoculation averages:				
Continued	1.2		27	
Changed	1.1		18	
Difference required for significance (5-percent level)	.1		4	

[1] 1, Less than 1 mm.; 2, 1 to 1.9 mm.; 3, 2 to 2.9 mm.; 4, 3 mm. or more.
[2] Nutrient solution high in nitrogen, phosphorus, and potassium supplied after inoculation of the plants and continued until the end of the experiment.
[3] No leaf spots or dead leaves occurred on uninoculated plants kept throughout the experiment on the same solution (continued treatment).

By averaging all the data for the different N levels it was found that the low- and medium-N plants in the continued group of experiment 1 had significantly smaller spots than high-N plants. In experiment 2 the leaf spots were significantly smaller on the low-N plants. The low-N plants in experiment 3 had significantly smaller leaf spots on the sixth leaf than the medium- and high-N plants. As a group the high-P plants had significantly smaller leaf spots than the low-P in experiments 1 (continued group) and 3. There was no significant difference in size of leaf spots on plants grown at the different P levels in experiment 2. On the average, in experiments 1 and 2 the low-K plants had significantly smaller leaf spots than the high-K ones. There were no significant differences in size of leaf spots on the sixth leaf of plants grown at the three K levels in experiment 3.

On the younger leaves (ninth above the cotyledons, experiment 3) on the differently treated plants there was very little difference in the size of leaf spots.

TABLE 3.—*Susceptibility of tomato leaves to Alternaria solani as influenced by different levels and combinations of nitrogen, phosphorus, and potassium nutrition, experiment 2*

[Each average based on 4 plants; 10 days after inoculation for size of leaf spots and 28 days after inoculation for dead leaves]

Nutrition treatment	Average class [1] for size of leaf spots	Leaves dead [2]
		Percent
Low nitrogen:		
Low phosphorus and low potassium	2.5	30
Low phosphorus and high potassium	3.0	41
High phosphorus and low potassium	2.8	35
High phosphorus and high potassium	3.0	48
High nitrogen:		
Low phosphorus and low potassium	3.8	31
Low phosphorus and high potassium	5.0	54
High phosphorus and low potassium	2.8	14
High phosphorus and high potassium	4.2	35
Difference required for significance (5-percent level)	1.4	13
Nitrogen averages:		
Low	2.8	38
High	3.9	34
Phosphorus averages:		
Low	3.6	39
High	3.2	33
Potassium averages:		
Low	2.9	28
High	3.8	45
Difference required for significance (5-percent level)	.7	7

[1] 1, Less than 1 mm.; 2, 1 to 1.9 mm.; 3, 2 to 2.9 mm.; 4, 3 to 3.9 mm.; 5, 4 mm. or more. All spots on the fifth leaf above the cotyledon were classified.

[2] No leaf spots or dead leaves occurred on uninoculated plants.

In experiment 1 (continued group) there was a significant interaction between N and K. At the low-K level the spots were significantly smaller than at the high-K level on the high-N and low-N plants, but not on the medium-N ones. In experiment 3 a significant interaction between N and P was evident on the sixth leaf above the cotyledons. At the low-P level the leaf spots were larger on the medium- and high-N plants than on the low-N ones, whereas at the high-P level there was little difference in size of leaf spots on the plants at different N levels. A significant interaction existed between N and P and N and K on the ninth leaf above the cotyledons in experiment 3.

Supplying plants previously low in N, P, and K with a high level of them at the time of inoculation retarded the development of leaf spots (table 2).

With one exception the leaf spots on the sixth leaf above the cotyledons were larger than those on the ninth leaf regardless of treatment (table 4). Leaf spots that were less than 1 mm. in diameter 13 days after inoculation increased in 21 days to a range of 1.2 to 2.1 mm., depending upon the nutrient solution supplied the plant. In the 34-day group there were no significant differences among the averages for the N and K levels. The high-P plants had significantly smaller leaf spots than the low-P ones.

TABLE 4.—*Susceptibility of tomato leaves of different ages to Alternaria solani as influenced by different levels and combinations of nitrogen, phosphorus, and potassium nutrition, experiment 3*

[Each average (10 days after inoculation) for columns 2 and 3 based on 4 leaves per plant, for column 4 on 3 leaves per plant (34 days after inoculation), and for column 5 on all leaves (34 days after inoculation)]

Nutrition treatment	Average class [1] for size of leaf spots on indicated leaf			Leaves dead [3]
	Sixth above cotyledon	Ninth above cotyledon	3 per plant [2]	
				Percent
Low nitrogen:				
Low phosphorus and low potassium	1.3	1.1	1.8	19
Low phosphorus and medium potassium	1.2	1.1	1.7	19
Low phosphorus and high potassium	1.2	1.0	1.5	23
High phosphorus and low potassium	1.0	1.0	1.2	12
High phosphorus and medium potassium	1.2	1.0	1.3	11
High phosphorus and high potassium	1.1	1.0	1.4	7
Medium nitrogen:				
Low phosphorus and low potassium	1.4	1.1	1.8	15
Low phosphorus and medium potassium	1.7	1.6	1.7	21
Low phosphorus and high potassium	2.0	1.2	1.8	30
High phosphorus and low potassium	1.6	1.0	1.5	18
High phosphorus and medium potassium	1.1	1.0	1.2	15
High phosphorus and high potassium	1.2	1.0	1.2	11
High nitrogen:				
Low phosphorus and low potassium	1.5	1.2	1.6	16
Low phosphorus and medium potassium	2.3	1.1	2.1	34
Low phosphorus and high potassium	1.8	1.2	2.1	24
High phosphorus and low potassium	1.1	1.0	1.2	4
High phosphorus and medium potassium	1.2	1.1	1.2	2
High phosphorus and high potassium	1.2	1.0	1.2	6
Difference required for significance (5-percent level)	.4	.2	.6	11
Nitrogen averages:				
Low	1.2	1.0	1.5	15
Medium	1.5	1.2	1.5	18
High	1.5	1.1	1.6	14
Difference required for significance (5-percent level)	.2	.1	.3	5
Phosphorus averages:				
Low	1.6	1.2	1.8	22
High	1.2	1.0	1.2	10
Difference required for significance (5-percent level)	.1	.1	.2	4
Potassium averages:				
Low	1.3	1.0	1.5	14
Medium	1.4	1.1	1.5	17
High	1.4	1.1	1.5	17
Difference required for significance (5-percent level)	.2	.1	.3	5

[1] 1, Less than 1 mm.; 2, 1 to 1.9 mm.; 3, 2 to 2.9 mm.; 4, 3 mm. or more.
[2] 13 days after inoculation 3 leaves per plant having spots less than 1 mm. in diameter were selected; they were classified for size 21 days later.
[3] No leaf spots or dead leaves occurred on uninoculated plants.

PERCENTAGE OF LEAVES DEAD

The smallest percentage of leaves dead in experiment 1 (continued group) was found on the high-N, high-P, high-K plants (table 2); in experiment 2 on the high-N, high-P, low-K plants (table 3); and in experiment 3 on the high-N, high-P, medium K-plants (table 4). In experiments 1 (continued group) and 2 the largest percentage of leaves dead occurred on the high-N, low-P, high-K plants and in experiment 3 on the high-N, low-P, medium-K plants. Representative plants grown on the 12 solutions of experiment 1 are shown in figures 4 to 6.

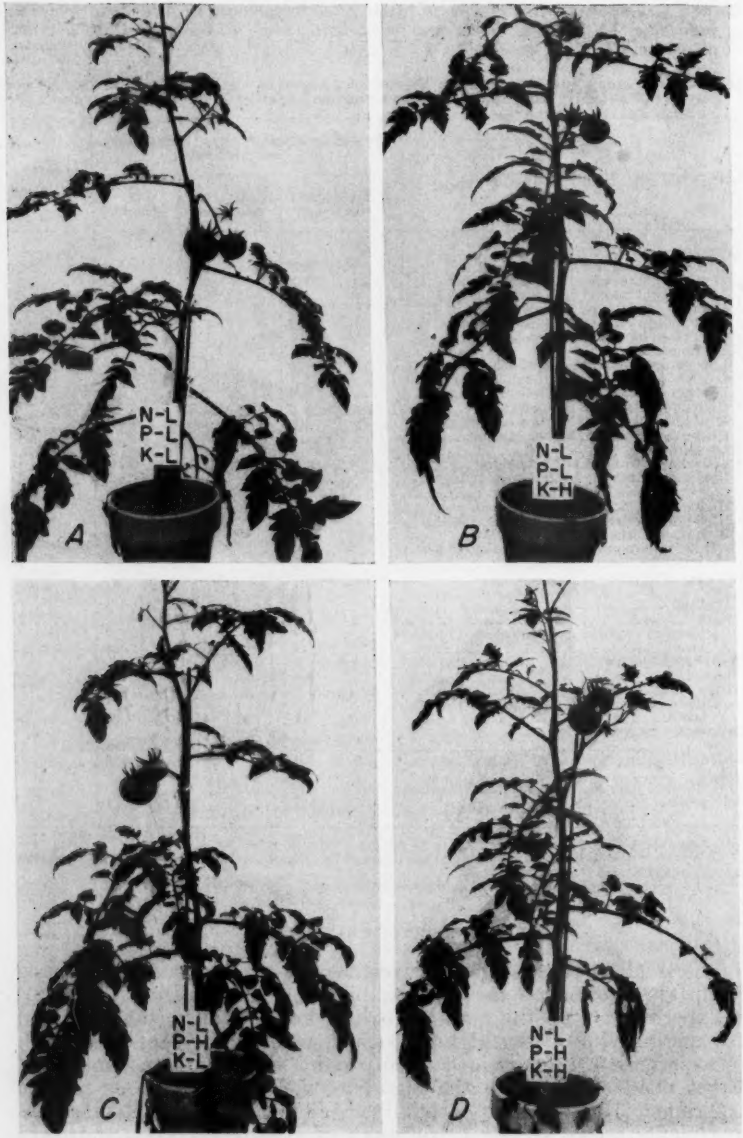

FIGURE 4.—Tomato plants grown at a low-nitrogen level, showing susceptibility of leaves to *Alternaria solani*, experiment 1: *A*, Low phosphorus and low potassium; *B*, low phosphorus and high potassium; *C*, high phosphorus and low potassium; *D*, high phosphorus and high potassium.

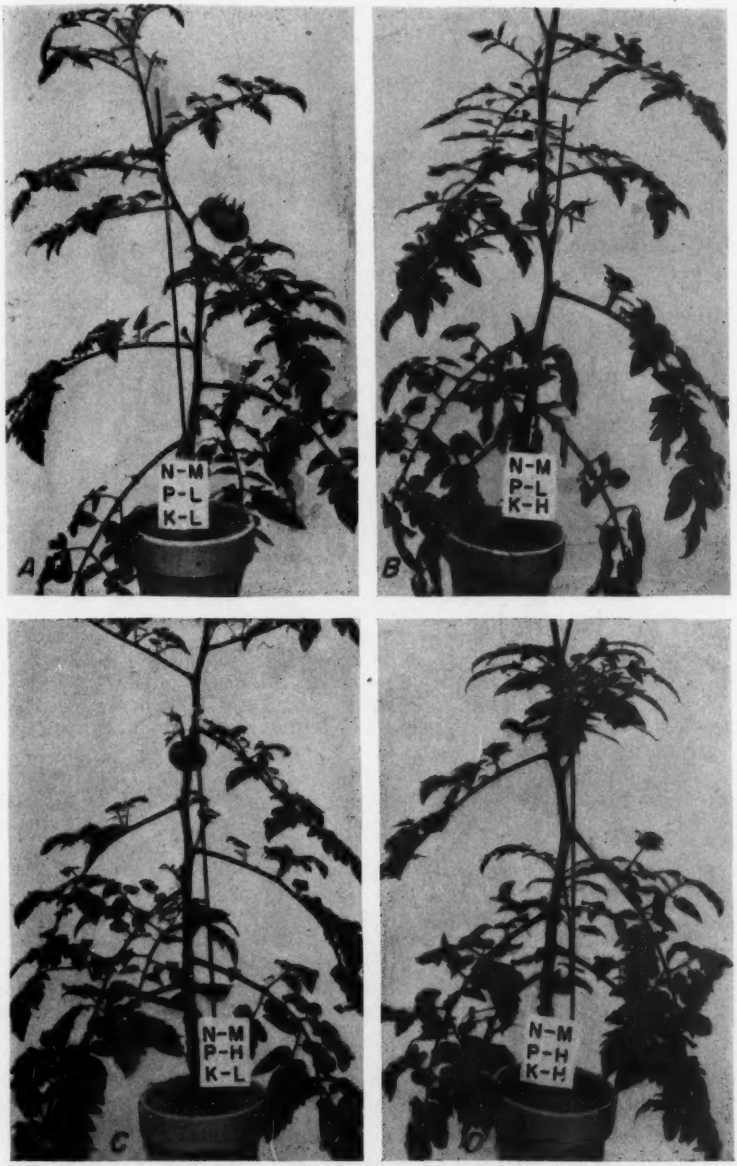

FIGURE 5.—Tomato plants grown at a medium-nitrogen level, showing susceptibility of leaves to *Alternaria solani*, experiment 1: *A*, Low phosphorus and low potassium; *B*, low phosphorus and high potassium; *C*, high phosphorus and low potassium; *D*, high phosphorus and high potassium.

FIGURE 6.—Tomato plants grown at a high-nitrogen level, showing susceptibility of leaves to *Alternaria solani*, experiment 1: *A*, Low phosphorus and low potassium; *B*, low phosphorus and high potassium; *C*, high phosphorus and low potassium; *D*, high phosphorus and high potassium.

By averaging the data for the different N levels it was found in all three experiments that there was no significant difference in the percentage of leaves dead. In experiment 1 (continued group) and experiment 3 the high-P plants had a significantly smaller percentage of leaves dead than the low-P ones. There were no significant differences in percentage of leaves dead at the different P levels in experiment 2. The low-K plants had a significantly smaller percentage of leaves dead than the high-K ones in experiments 1 (continued group) and 2. In experiment 3 there were no significant differences in percentage of leaves dead at the different K levels.

In the continued group of experiment 1 and in the other experiments there was a significant interaction between N and P. In experiments 1 and 2 on the low-N plants there was more defoliation at the high-P level than at the low-P, whereas the reverse occurred at the other N levels. In experiment 3 the percentage of leaves dead, although less on the high-P than on the low-P plants at all N levels, was much smaller at the high-N level than at either the medium- or the low-N level. In experiment 1 there was a significant interaction between N and K. At the high-K level there was a proportionately greater percentage of leaves dead on the low-N plants than on the medium- and high-N ones.

Supplying plants previously low in N, P, and K with them at the time of inoculation reduced the percentage of leaves dead (table 2).

FIELD RESULTS

On August 13 there were no significant differences in percentage of leaves dead on plants grown on the different soil types, so that averages for the two soils can be compared in studying results for that date. The most defoliation occurred on the low-N, low-P, high-K plots and the least on the high-N, high-P, low-K ones (table 5).

On the average, significantly less defoliation occurred on the high-N plants than on the low-N ones. It is recognized that part of the defoliation on the low-N plants may have been due to nutritional effects aside from those involving attacks by *Alternaria*. There were no significant differences between P or K levels.

Records taken September 2 show that the amount of defoliation, 72 to 90 percent, is so great that any slight differences that existed are of no practical significance.

In the field no practical differences in the percentage of leaves dead existed between the treatments late in the season. This was particularly true on the Brookston silty clay loam of high fertility, where the plants were large and conditions were favorable for the increase of the fungus. The greater defoliation on the low-N plants, particularly noticeable early in the season, may have been due more to the early development of a relatively heavy crop of fruit than to the direct effect of nitrogen on disease development. This might also be the explanation for the greater defoliation of the high-P than of the low-P plants late in the season.

TABLE 5.—*Susceptibility of field-grown tomato plants to infection by Alternaria solani on Crosby and Brookston soil series as influenced by different levels and combinations of nitrogen, phosphorus, and potassium nutrition*

[Each soil-type average based on 4 replicates of 6 plants each]

Nutrition treatment	Leaves dead (Aug. 13) when grown on indicated soil type			Leaves dead when grown on Crosby loam (Sept. 2)	Early yield per acre (Aug. 29) [1] when grown on indicated soil type		
	Crosby	Brookston	Both		Crosby	Brookston	Both
	Percent	*Percent*	*Percent*	*Percent*	*Tons*	*Tons*	*Tons*
Low nitrogen:							
Low phosphorus and low potassium	32	32	32	84	8.0	7.6	7.8
Low phosphorus and high potassium	34	33	33	72	8.4	8.8	8.6
High phosphorus and low potassium	26	29	28	85	9.6	9.4	9.5
High phosphorus and high potassium	24	30	27	90	9.0	8.2	8.6
High nitrogen:							
Low phosphorus and low potassium	18	17	17	76	6.3	6.8	6.6
Low phosphorus and high potassium	22	22	22	78	7.2	7.0	7.1
High phosphorus and low potassium	12	18	15	85	6.2	7.9	7.0
High phosphorus and high potassium	18	28	23	74	6.3	7.4	6.8
Difference required for significance (5-percent level)	13	13	9	8	2.3	2.3	1.6
Nitrogen averages:							
Low			30	83			8.6
High			19	78			6.9
Phosphorus averages:							
Low			26	78			7.5
High			23	84			8.0
Potassium averages:							
Low			23	82			7.7
High			26	78			7.8
Difference required for significance (5-percent level)			5	4			1.1

[1] No additional yields were obtained.

DISCUSSION

The data presented show that the degree of injury caused by *Alternaria solani* infection of tomato foliage was influenced by the supply of N, P, and K. The reactions to the various levels and combinations of these elements differed somewhat in each of the three greenhouse experiments. The differences may have been due to variations in the composition of the plant caused by fluctuations in the amount of sunlight during the growing period, in the environment during the period of disease development, or in the composition of the nutrient solutions. In experiments 1 and 2 the supply of P was not adequate at times to maintain as high a level as was desired in the high-N, high-P, high-K plants.

Plants receiving certain treatments reacted to infection similarly in all three series as well as in several experiments not described herein. The plants grown on solutions medium to high in N, high in P, and low to high in K were injured least by *Alternaria solani*. In several instances the low-N, low-P, low-K plants were also damaged little. Plants grown on solutions of medium- to high-N, low-P, medium- to high-K level were usually the most severely injured.

There probably would have been fewer dead leaves on the high-N, high-P plants in experiments 1 and 2 if a higher level of P had been maintained throughout the experiment.

In general the susceptibility of the plants as indicated by the size of the leaf spots agreed with that indicated by the percentage of leaves dead. The susceptibility of the plants as measured by the number and size of stem cankers (data not presented) did not correlate so closely.

In all three series the percentages of leaves dead indicated that there was a significant interaction between N and P. The expression of the interaction, however, was not always consistent. In experiments 1 (continued group) and 2 at the low-N level the high-P plants had more defoliation than the low-P plants. In experiment 3 the reverse occurred. In every case the high-N, low-P plants had more defoliation than those grown with other N and P combinations and the high-N, high-P plants always had the least. Observing the over-all effects of N, P, and K, respectively, obscures the effects of interaction between these elements.

The mechanism responsible for the differences in reaction to disease development is unknown. The rapid collapse of leaf tissue about the point of infection on the susceptible plants suggests that a toxin may be formed in greater amounts in leaf tissue of the type developing on plants of a particular nutrient condition or may diffuse more rapidly through it. Whipple (*8*) reported that a toxin is produced by *Alternaria solani* and diffuses into the plant tissue for 12 cm. or more in advance of the mycelium. The apparent effect of toxin ahead of the mycelium has also been noted by Thomas.[4]

The degree of resistance exhibited under the controlled conditions in the greenhouse would probably be of limited value in the field. Infection of new growth in the field occurs at such a rapid rate under suitable weather conditions that any differences that might exist in the sizes of leaf spots would be concealed by the abundance of infection. However, plants with adequate nutritional supply are able to continue growing and develop new shoots in the center throughout the season and thus protect the fruit from sun injury. This type of protection is satisfactory until a period of weather ideal for heavy infection again occurs.

SUMMARY

Varying the amounts and combinations of N, P, and K in sand-culture greenhouse tests affected the response of tomato plants to infection by *Alternaria solani*.

By averaging all the data for the different P levels it was found that in two of the three experiments the plants grown at the high level of P, irrespective of N and K levels, had smaller leaf spots and a significantly smaller percentage of leaves dead than the plants grown at the low-P level.

By averaging all the data for the different N levels it was found that in each of the three experiments plants grown at the low level of N, irrespective of P and K levels, had significantly smaller leaf spots than those at the high-N level. There was no significant difference in the percentage of leaves dead on the plants on the different N levels.

On the average, plants grown at the low K level, irrespective of N and P levels, had a significantly smaller percentage of leaves dead and smaller leaf spots than those grown at the high level in two of the three series.

[4] THOMAS, H. R. COLLAR-ROT INFECTION ON DIRECT-SEEDED TOMATOES. U. S. Bur. Plant. Indus., Plant Dis. Rptr. 24 (1): 8–10. 1940. Processed.]

A significant interaction existed between N and P in the percentage of leaves dead in all three greenhouse experiments. In every case the high-N, low-P plants had the largest percentage of leaves dead and the high-N, high-P plants the smallest.

Plants grown on solutions medium to high in N, low in P, and medium to high in K were usually the most susceptible to infection as expressed by the development of leaf spots and percentage of leaves dead. The most resistant plants were grown on solutions medium to high in N, high in P, and low to high in K.

The group of plants that received a nutrient supply high in N, P, and K at the time of inoculation had significantly smaller leaf spots and percentage of leaves dead than the group which continued to receive solutions deficient in N, P, and K after inoculation.

The leaf spots were larger on the older leaves than on the younger ones, regardless of the apparent nutritional condition of the plants.

A limited field fertilizer test in Indiana indicated little if any difference in the disease resistance of plants grown at different levels of N, P, and K and with combinations of them.

LITERATURE CITED

(1) Barratt, R. W., and Richards, M. C.
1944. alternaria blight versus the genus lycopersicon. N. H. Agr. Expt. Sta. Tech. Bul. 82, 24 pp., illus.

(2) Horsfall, J. G., and Heuberger, J. W.
1942. causes, effects and control of defoliation on tomatoes. Conn. (State) Agr. Expt. Sta. Bul. 456, pp. [181]–223, illus.

(3) Moore, W. D., and Thomas, H. R.
1943. some cultural practices that influence the development of alternaria solani on tomato seedlings. Phytopathology 33: 1176–1184, illus.

(4) Snedecor, G. W.
1946. statistical methods applied to experiments in agriculture and biology. Ed. 4, 485 pp., illus. Ames, Iowa.

(5) Thomas, H. R.
[1940.] nitrogen nutrition of tomato seedling affects susceptibility to collar rot. Ind. Agr. Expt. Sta. Ann. Rpt. (1938–39) 52: 56.

(6) ———
1943. a nonchromogenic sporulating variant of alternaria solani. Phytopathology 33: 729–731, illus.

(7) Thornton, S. F., Conner, S. D., and Fraser, R. R.
1936. the use of rapid chemical tests on soils and plants as aids in determining fertilizer needs. Ind. Agr. Expt. Sta. Cir. 204, 16 pp., illus. (Revised.)

(8) Whipple, O. C.
1938. macrosporium seedling blight of tomatoes. Wis. Univ. Sums. Doctoral Diss. 3: 65–66.

REACTION OF ALFALFA VARIETIES, SELECTIONS, AND HYBRIDS TO ASCOCHYTA IMPERFECTA[1]

By L. P. REITZ, *agronomist, Kansas Agricultural Experiment Station;* C. O. GRANDFIELD, *associate agronomist, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture;* M. L. PETERSON, G. V. GOODDING, M. A. ARNESON, formerly *graduate research assistants;* and E. D. HANSING, *associate plant pathologist, Kansas Agricultural Experiment Station*

INTRODUCTION

Black stem caused by *Ascochyta imperfecta* Peck is an important disease of the leaves and stems of alfalfa (*Medicago sativa L.*). It is especially destructive in cool, wet seasons and in humid regions. In Kansas it causes greatest damage to the spring growth. In years of heavy infection, the yield may be reduced and the feeding quality lowered, especially as defoliation progresses. In severe cases the stands of alfalfa are made thin or killed entirely, but this is not the usual result from infection at this station. The disease has been reported in many of the alfalfa-growing States of the United States and in some other countries.

Peterson and Melchers [2] reviewed the literature and reported research on the characteristics of the disease, identification of the causal organism, and methods used in artificial inoculation.

Early symptoms of black stem are small, dark-brown or black spots on the leaves and stems. As the lesions enlarge and coalesce the leaves become chlorotic and drop from the plant. Stem lesions turn black and coalesce, causing the stem surface of infected areas to blacken and sometimes to die. The portions nearest the ground usually are most severely infected. Greenhouse inoculations with spore suspensions of the fungus produced typical lesions on the leaves and stems, but the most uniform infection was obtained on the leaves.

It is the purpose of this paper to present the data on genetic differences in susceptibility to the fungus observed in experiments conducted at Manhattan, Kans., during the years 1938 to 1943, inclusive.

MATERIAL AND METHODS

Observations on susceptibility of varieties and strains of alfalfa to black stem were made in the field and greenhouse. In the field, plots sown for yield determinations or demonstrations were used. Green-

[1] Received for publication September 2, 1947. Contribution No. 383 from the Department of Agronomy and No. 484, serial No. 394, from the Department of Botany, Kansas Agricultural Experiment Station, in cooperation with the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

[2] PETERSON, M. L., and MELCHERS, L. E. STUDIES ON BLACK STEM OF ALFALFA CAUSED BY ASCOCHYTA IMPERFECTA. Phytopathology 32: 590–597, illus. 1942.

house plantings were made in unglazed clay pots. Usually one plant was grown in each pot. The plants were inoculated by spraying them with a suspension of spores, after which they were placed in a canvas-covered moist chamber and kept there for 72 hours.

Considerable time was spent in finding a suitable method of scoring the plants for reaction to black stem. Classes of resistance formed an intergrading series and immunity from disease was not observed in any variety or strain. Several somewhat different methods were used, but high correlations in the results of the different methods were noted. In most of the greenhouse tests disease scores for individual plants were determined by ratings on the 0 to 5 or 0 to 10 basis. Plants were scored for (1) number of leaf lesions, (2) size of leaf lesions, (3) defoliation, (4) petiole lesions, and (5) stem lesions. These can be transferred to a basis of 100 by multiplying each score by 10 or 20 depending upon the basis of scoring and the numbers averaged for a plant score. Typical leaves showing degrees of infection are shown in figure 1.

FIGURE 1.—Typical leaves of alfalfa showing grades of black stem infection on a scale of 0 to 5. Leaves in upper row show increasing numbers of lesions; those in lower row show different sizes of lesions.

By artificial inoculation and with a moist chamber, many plants grown in the greenhouse could be tested. When this was done, each group of plants was accompanied by four check plants from a clonally propagated plant of Ladak alfalfa. By scoring the check plants an index of infection could be calculated and different groups compared. This was the most satisfactory means of comparing lots inoculated at different times. A sample calculation follows:

(1) Number of leaf lesions	6×10=60
(2) Size of leaf lesions	4×20=80
(3) Defoliation	7×10=70
(4) Petiole lesions	3×20=60
(5) Stem lesions	1×20=20
Total	290

290÷5=58 the average plant score.

If the score of check plants was 48, the index number of infection of this plant would be 58÷48×100 or 121.

Richards [3] devised a system of scoring plants in the field. A sample consisted of all the stems which were within a ring cast into a plot. Enclosed stems were separated into six classes depending upon the degree of severity of the disease, with 1 as the class of least severity. The number of stems was multiplied by the class number and the sum total thus derived was divided by the total number of stems collected. This method was satisfactory under field conditions when infection on the stems was heavy, but was not so good for mild infection or during early stages of development. A modified system was devised in the present study which gave weight to leaves and stems separately. Such values could be combined for a plant score, and scores could be changed to percentages as desired.

Isolations of the fungus were made from several collections to study cultural characteristics and differences in pathogenicity.

The analysis of variance, in conjunction with associated tests of significance, afforded the basis for most of the statements contained in this paper on statistical differences between units or treatments. Several other statistical methods also were used to reduce the large masses of data recorded. In general, these are mentioned when different experiments are discussed.

EXPERIMENTAL RESULTS

VARIETAL REACTION UNDER FIELD CONDITIONS

Johnson and Valleau [4] indicated that resistance to black stem was inherent in some varieties of alfalfa. Toovey, Waterston, and Brooks [5] gave the results of black stem readings on alfalfa strains in which "the strains were placed in the following order of decreasing susceptibility in 1935: (1) Medanos, (2) English grown and Grimm, (3) Provence, Marlborough and Hungarian." Richards [3] made detailed readings on 44 varieties during a severe epidemic in 1933, and found the disease to be least severe in Ladak and most severe in a French introduction. Introductions from Russia and Turkistan were severely infected; Grimm and Hardigan were less damaged. Peterson and Melchers [6] reported varietal and species differences.

---

[3] RICHARDS, B. L. REACTION OF ALFALFA VARIETIES TO STEM BLIGHT. Phytopathology 24: 824–827, illus. 1934.

[4] JOHNSON, E. M., and VALLEAU, W. D. BLACK-STEM OF ALFALFA, RED CLOVER AND SWEET CLOVER. Ky. Agr. Expt. Sta. Bul. 339: 55–82, illus. 1933.

[5] TOOVEY, F. W., WATERSTON, J. M., and BROOKS, F. T. OBSERVATIONS ON THE BLACK-STEM DISEASE OF LUCERNE IN BRITAIN. Ann. Appl. Biol. 23: 705–717, illus. 1936. (See p. 707.)

[6] See footnote 2, p. 307.

Varietal differences in susceptibility were found in preliminary trials at Manhattan in 1939 from four sets of data involving three methods of making disease readings. Differences between varieties in each experiment were statistically significant, indicating the possibility of breeding for resistance. Turkistan varieties were more susceptible than Ladak or Kansas Common in these trials.

The spring of 1942 was exceptionally cool and wet; thus climatic conditions were favorable both for plant growth and for infection with black stem in the field. Readings were made on 66 strains of alfalfa planted in 20-foot rows with two replications in the uniform nursery on the agronomy farm at Manhattan. The nursery contained improved strains from Rhode Island, New Jersey, New York, Michigan, Wisconsin, Nebraska, Kansas, and Colorado as well as some of the standard varieties, such as Ladak, Kansas Common, Grimm, Meeker Baltic, Orestan, Hardistan, Dakota Common, and Arizona Chilean. In addition, a few foreign introductions were grown. Notes on black stem were taken on April 30, or about a month before the crop was cut for hay. At that time black stem was the predominant disease, but 4 weeks later yellow leaf blotch caused by *Pseudopeziza jonesii* Nannf. was more abundant than black stem. Severe defoliation had occurred from the time of the first reading and a large share of this was attributed to black stem. Analysis of variance of the black stem readings showed that the variation due to strains was highly significant, far exceeding the 1-percent level, thus indicating a real difference among the strains. A–169 from Nebraska, A–155 from Rhode Island, and 128 and A–131 from Wisconsin were the most resistant strains according to these readings. All of the foreign introductions and Arizona Chilean were very susceptible. Of the standard varieties, Grimm and Dakota Common ranked highest in resistance.

OBSERVATIONS IN GREENHOUSE TESTS

A comprehensive test of 45 artificially inoculated plants of each of 10 varieties was made in the greenhouse. The purpose of this experiment was (1) to test the reliability of readings on artificially inoculated plants, and (2) to determine whether improvements could be made by selecting plants in this way. The 10 varieties were Turkistan 86696, Turkistan 19304, Ladak, Kansas Common, Kansas Common Selection, Grimm, Hairy Peruvian 22486, *Medicago falcata* F. C. 30114, *Medicago ruthenica* F. P. I. 190365, and Semipalatinsk F. C. 22613. Seed was planted in flats in the greenhouse on May 17, and on June 22 the seedlings were transplanted to rows in the irrigated nursery. On October 2, 5 plants from each variety were dug, transplanted into 7-inch pots, and brought into the greenhouse. Five days later a second group of 50 plants was brought into the greenhouse. This procedure was repeated every 5 days until 450 plants had been potted.

By November 28 the first group of plants was of sufficient size to be inoculated. Inoculations were made at regular intervals so that the foliage on all groups of plants was approximately the same age when the plants were inoculated. Two readings were made on each plant. The first was made 8 to 10 days after inoculation and the second 5 days later. A summary of the readings is given in table 1. The disease score for the whole plant is the sum of the scores for

stems and leaves estimated on a 0 to 10 basis. Clonally propagated check plants were not used in this test.

TABLE 1.—*Average disease readings and rank of 45 plants from each of 10 alfalfa varieties, showing coefficients of variation for size and number of lesions*

Variety	Whole plant		Score of—		Size of lesions		Number of lesions	
	Score	Rank	Stems	Leaves	Score	Coefficient of variation	Score	Coefficient of variation
Medicago ruthenica	2.38	1	0.42	1.96	2.11	49.7	1.80	57.5
Semipalatinsk	3.39	2	.58	2.81	3.18	36.9	2.44	45.8
Ladak	3.47	3	.53	2.94	3.33	27.1	2.56	54.4
Grimm	3.93	4	.78	3.15	3.44	24.4	2.87	47.4
Medicago falcata	4.08	5	.91	3.17	3.44	34.2	2.89	49.1
Turkistan 19304	4.36	6	.80	3.56	3.58	28.3	3.53	36.5
Turkistan 86696	4.37	7	.87	3.50	3.71	26.1	3.29	41.8
Kansas Common	4.71	8	1.15	3.56	3.80	26.7	3.31	42.1
Kansas Common Selection	4.93	9	1.35	3.58	3.93	23.6	3.22	45.9
Hairy Peruvian	6.19	10	2.02	4.17	4.44	17.0	3.89	26.9
Average	4.08	-------	.94	3.24	3.50	29.4	2.98	44.7

In this experiment, the disease scores ranged from 2.38 for *Medicago ruthenica*, the most resistant variety, to 6.19 for Hairy Peruvian, which was extremely susceptible. The Turkistans and Kansas Commons ranked higher than average in susceptibility. The fact that Kansas Common scored higher in susceptibility than Turkistan is important, since disease readings in the field under natural conditions for infection have consistently shown Turkistan types to be the more heavily diseased. These results suggest that the two varieties may react differently under conditions of natural and artificial infection. Differences in disease readings for varieties were highly significant.

Stems showed rather poor infection from artificial inoculation; however, differences between variety means were significant beyond the 1-percent point. Leaf readings showed heavier infections than those for stems with a wider range in scores. The rank of the different varieties for stem and leaf scores corresponded very closely. Thus it appeared that factors conditioning stem infection were the same as those conditioning leaf infection.

The leaf score was obtained by giving equal but separate consideration to size of lesions and number of lesions. There were two reasons for considering these two factors independently: (1) A plant with few but very large lesions would be damaged as severely as a plant with more numerous but small lesions and (2) preliminary inoculations had shown that plants varied considerably in respect to size and number of lesions. The score for size of lesions ranged from 2.11 on *Medicago ruthenica* to 4.44 on Hairy Peruvian. The differences were highly significant.

The score for number of lesions on the leaves ranged from 1.80 on *Medicago ruthenica* to 3.89 on Hairy Peruvian and the differences between variety means were highly significant.

Comparison of the ranking of the various varieties for size and number of lesions indicated a close relationship in general, although

Turkistan 19304 ranked sixth in size of lesions and ninth in number of lesions while Kansas Common Selection ranked ninth in size of lesions and sixth in number of lesions. These results indicate that there may be two plant characteristics for resistance of leaves to black stem disease, one restricting the entrance of the organism, the other inhibiting its growth once it is inside.

PLANT VARIATION WITHIN VARIETIES

There were wide variations in disease readings within a variety. For example, readings for Kansas Common ranged from 1.5 to 9.0, Turkistan 86696 from 2.0 to 8.0, and Ladak from 1.5 to 8.0. This range included several sources of variation, a part of which was considered heritable. Coefficients of variability were calculated to indicate variation within varieties with respect to size and number of lesions (table 1).

Hairy Peruvian showed the least variability for size of lesions, i. e., it approached complete susceptibility. Kansas Common Selection, Grimm, Turkistan 86696, Kansas Common, Ladak, and Turkistan 19304 followed in increasing order of variability but with only a small range from low to high. *Medicago falcata*, Semipalatinsk, and *M. ruthenica* were considerably more variable. The coefficients for number of lesions also ranked Hairy Peruvian as least variable and *M. ruthenica* as most variable.

REACTION OF 30 PEDIGREE LINES FROM KANSAS COMMON

Ten plants from each of 30 bacterial wilt-resistant selections from Kansas Common were potted in the field and brought into the greenhouse in November 1942. These were young plants from seed planted on August 30. When the plants became 8 to 10 inches high they were selected at random on the basis of height only and inoculated with the fungus. Readings were made in the usual manner, and the scores and indices were calculated. In this case a Turkistan clone was used as the standard check. Considerable variability within lines was found. Since these lines were from open-pollinated plants. much variability was expected because of the well-known heterozygosity in alfalfa. The plants were cut back after the readings had been made on the first inoculation. Many of the plants were blossoming at that time. Four weeks later these plants were inoculated again and readings were made. A highly significant difference existed between inoculations, and the lines were significantly different. Kansas Common line 1–102–5 had the lowest average score of 82 for 7 plants.

Readings from the second inoculation exceeded those from the first in 23 of the 30 lines tested. In the other 7 lines the reverse was true. It is difficult to explain the significant variation between inoculations, as all plants were of the same age and received the same treatment. Some of the variation may have been due to variation in readings and some to the difference in recovery of the plants after cutting, as some plants recovered faster than others. The root reserves may have been higher in the plants when inoculated the second time than the first, as the majority of the plants were blooming or about to bloom

when cut back after the first inoculation. Therefore, differences in root reserves would be expected. Temperature likewise may have been a contributing factor since greenhouse temperatures were higher during the early part of the season than later. Other factors not known at present may also have contributed to this difference between inoculations.

Analysis of variance showed that significant differences were present between lines, but there was so much variation within lines that the 22 highest ranking ones did not differ significantly.

REACTION OF PLANT SELECTIONS AND INBRED LINES

Parental stocks for inbreeding were selected from plants used in the variety tests reported above with the exception of the 30 bacterial wilt-resistant selections. At least 1 resistant and 1 susceptible plant were chosen from each variety. The terms "resistant" and "susceptible" as used here, refer to the relative resistance of plants within each variety. Thus, a resistant plant from a relatively susceptible variety might have been more susceptible to the disease than a susceptible plant from a more resistant variety.

The selected plants were self-pollinated in the greenhouse in an insect-free room. Enough seed was gathered from each plant to insure a progeny class of at least 27 plants. The seeds were scarified and planted in flats. When seedlings had attained about 4 inches of growth, they were transferred to 6-inch pots, where they were left throughout the tests. Clonally propagated check plants were used as standards for comparison in all inoculation tests.

Inbred plants were allowed to develop a vigorous growth and were selected for inoculation when they were 10 to 12 inches in height. Plants were chosen at random throughout all of the varieties used, with the size of the plant as the only characteristic considered in making selections for inoculation.

A detailed description of the results obtained in testing the inbred plants from the selected parents follows:

Kansas Common variety.—Two plants were used as parents from which inbred lines were established in the variety Kansas Common. Kansas Common No. 6 was a resistant plant and Kansas Common No. 18 was susceptible. Twenty-seven inbred plants from each of these parents were tested. Their plant scores were derived and each plant was given an index of infection in terms of the check plants' score. The two progenies were compared by the use of analysis of variance; the results are presented in table 2.

The means of each group clearly indicate that the progeny of No. 6 was significantly more resistant than the progeny of No. 18. The analysis of variance substantiated this assumption, for it showed that highly significant differences existed between the two inbred lines. The standard deviations of the two groups showed that considerable variation occurred in each population.

Ladak variety.—Four inbred lines were developed in the variety Ladak. Of the four parent plants, No. 11 was resistant and the other three plants, Nos. 20, 5, and 15, were only moderately resistant. On the basis of inheritance, the inbred lines on the average were expected to resemble their parents in resistance. Table 2 shows the differences

that occurred between the progeny of the resistant plant and the progenies of each of the susceptible plants. These data demonstrate the resistance of selection 11 and the similarity of the three progenies from the moderately resistant plants.

TABLE 2.—*A summary of the data on resistance to black stem in the inbred progenies of resistant and susceptible plant selections from 5 varieties and strains of alfalfa*

Variety and selection number	Inbred progenies		Standard deviation
	Mean	Difference	
Kansas Common:			
No. 6	101.3		37.9
No. 18	147.3	46.0**	29.0
Ladak:			
No. 11	89.9		20.4
No. 20	111.6	21.7**	24.9
No. 11	89.9		20.4
No. 5	118.3	28.4**	32.0
No. 11	89.9		20.4
No. 15	105.8	15.9**	22.7
No. 20	111.6		24.9
No. 5	118.3	6.7	32.0
No. 20	111.6		24.9
No. 15	105.8	5.8	22.7
No. 5	118.3		32.0
No. 15	105.8	12.5	22.7
Turkistan:			
No. 36	128.1		10.6
No. 16	113.3	14.8	32.9
No. 36	128.1		10.6
No. 40	144.0	15.9**	18.6
No. 16	113.3		32.9
No. 40	144.0	30.7**	18.6
Hairy Peruvian:			
No. 33	148.8		20.1
No. 5	142.2	6.6	32.6
No. 33	148.8		20.1
No. 13	163.4	14.6*	20.7
No. 5	142.2		32.6
No. 13	163.4	21.2**	20.7
Kansas Common Selection:			
No. 1–9	113.0		20.1
No. 1–6	131.7	18.7*	30.4
No. 1–9	113.0		20.1
No. 1–13	96.8	16.2**	18.8
No. 1–6	131.7		30.4
No. 1–13	96.8	34.9**	18.8

*Significant at 5-percent level.
**Significant at 1-percent level.

Turkistan variety.—Three Turkistan plants were selected for inbreeding. Two of them, Nos. 36 and 16, were moderately resistant, whereas the third one, No. 40, was very susceptible. The progenies of the 2 resistant plants maintained that resistance and showed no significant difference between groups. The progenies from the resistant parents were both significantly more resistant than the

inbreds developed from the susceptible plant No. 40. From Turkistan No. 36 there were only 21 inbred plants, 33 from No. 16, and the usual 27 from No. 40. The data summarized in table 2 indicate that inbreeding in the Turkistan variety results in the production of lines that react, as a group, much the same as their parents

Hairy Peruvian variety.—Three plants were selected for inbreeding from the very susceptible variety, Hairy Peruvian. Two of these plants, Nos. 33 and 5, were slightly resistant, and the third, No. 13, was highly susceptible. No significant difference was observed between the progenies of the two slightly resistant parents, as is shown in table 2, but they were significantly more resistant than the progeny of the susceptible parent, No. 13.

Kansas Common Selection.—Three plants of Kansas Common Selection were utilized for inbreeding. These three plants were classed as resistant, moderately resistant, and susceptible. The plant listed as susceptible produced inbred progeny with a higher level of resistance than either of the progenies of the supposedly resistant parents. Apparently the original classification of this plant was in error. The progeny of the plant listed as resistant was significantly more resistant than the progeny of the moderately resistant plant, demonstrating that in one instance, at least, this variety behaved as the other varieties did. On the other hand, some discrepancies were foreseen from the outset, since in early selection work no method was employed to correct for variations that arose between different dates of inoculation. Table 2 shows a summary of the data and comparisons of the differences that existed between the progenies of the plant selections.

Medicago falcata.—Two strains of the species *Medicago falcata* were utilized in the inbreeding studies. Both strains had probably been intercrossed to some extent with common alfalfas, but *M. falcata* features were evident in the parental selections, and a high degree of disease resistance, characteristic of the species, prevailed.

Medicago falcata No. 39.—A very resistant *M. falcata* plant was successfully inbred and its progeny proved to carry the same high level of resistance. The mean of the group was 81.61, the highest average resistance of any inbred group tested. The variation within this group was also low, as indicated by a standard deviation of ± 20.9.

Semipalatinsk No. 43.—Only one Semipalatinsk plant produced enough self-fertilized seed to permit the establishment of an inbred line for testing. This plant was rather resistant, and its resistance was carried on in the inbred population, as indicated by the mean, 107.7, of that group. The standard deviation ± 30.4 demonstrated considerable variation to exist within the line.

Thus, in all of the varieties and species represented in the first generation of inbreeding, there was a significant tendency for the inbred population from each parent plant to react to black stem in the same manner as the parent had reacted. One exception has been discussed. This tendency in an open-pollinated, heterozygous plant can be explained best on an inheritance hypothesis, for in such a plant, if inheritance were not involved, it is very probable that the observed

correlations of resistance in parent and progenies would not have existed.

Second-generation inbred progenies were obtained from nine of the 18 original plants mentioned above. The others failed to set seed or to produce viable seedlings for various reasons. Comparisons of infection from inoculations made in the greenhouse are shown in table 3. A correlation coefficient of 0.716, significant at the 5-percent level, was obtained between the indices of first- and second-generation progenies, thus further corroborating the evidence secured on first-generation inbreds.

The variation was lower in the S_2 generation than in the S_1. This was true in all but one strain tested and would be expected since homozygosity increases as inbreeding proceeds. The average standard deviation for the S_1 generation was 30.0 as compared with 20.7 in the S_2 generation. Thus inbreeding caused a large reduction in variability. All the variation was not due to the heterozygous nature of a line, as some occurred from the reading method and inoculation technique. This was shown by the variability among the four clonal check plants used with every inoculation. Only on rare occasions would the scores of these clones be identical. In every case, however, the variation between the check plants was small in comparison to the variation within a strain.

TABLE 3.—*Infection means and standard deviations on 9 lines for S_1 and S_2 generations*

Variety	Line	S_1 generation		S_2 generation	
		Mean	Standard deviation	Mean	Standard deviation
Ladak	G	101.8	24.9	81.8	14.5
Kansas Common	B	104.6	37.9	97.7	22.3
Semipalatinsk	J	108.7	30.4	92.1	18.3
Kansas Common	C	114.7	29.0	109.4	17.8
Ladak	H	119.9	32.0	124.3	22.0
Turkistan	N	120.5	32.9	133.2	16.7
Kansas Common Selection	L	124.5	20.1	105.1	27.4
Kansas Common Selection	K	126.2	30.4	116.7	28.0
Hairy Peruvian	D	134.9	32.6	117.0	19.5
Average		117.3	30.0	108.6	20.7

HYBRID STUDIES

The parents in the first cross studied were the highly susceptible inbred plant Hairy Peruvian No. 33 as the male parent and the very resistant *Medicago falcata* No. 39 inbred plant as the female parent. Thirty-three first-generation plants were developed from this cross and tested. The mean of the hybrid group was 93.85, denoting a very high degree of resistance. The standard deviation of ± 16.8 indicated low variability in the group. This suggested that the resistance of the *M. falcata* parent was dominant. A reciprocal cross was made in order to check the results. Only four first-generation plants developed for testing. All four were resistant, however, and their mean was 101.7. Thus, insofar as could be judged from these data, resistance also was dominant in the reciprocal cross.

An interesting comparison was noted between the hybrid population and the inbred progeny groups from each of the parents. The mean of the resistance of all the plants in the Hairy Peruvian No. 1 inbred line was 148.8, whereas the mean of the *M. falcata* No. 1 line was 81.61. The mean of the hybrid population from these parents was nearly as low as that of the resistant *M. falcata* No. 39 inbred line, for it was 93.85. Also, the uniformity characteristic of a first-generation hybrid population from a cross involving dominance was observed, for the standard deviation of the group was very low, ± 16.8, as compared with the deviations of the inbred groups from the same parents (Hairy Peruvian No. 33, $s = \pm 20.1$; *M. falcata* No. 39, $s = \pm 20.9$).

The infection data for 10 crosses of open-pollinated plants are shown in table 4. Each cross produced both resistant and susceptible

TABLE 4.—*Means of infection and number of plants in the F_2 generation of the crosses of open-pollinated plants*

Cross No.	Varieties crossed	Number in F_2	Mean
[1] 1	Hairy Peruvian × *Medicago falcata*	18	96
2	Kansas Common × Semipalatinsk	10	131
3	Hairy Peruvian × Kansas Common	104	112
4	*Medicago falcata* × Hairy Peruvian	10	102
5	Kansas Common × Hairy Peruvian	14	85
6	Ladak × Hairy Peruvian	49	104
7	Semipalatinsk × Ladak	126	93
8	Ladak × Hairy Peruvian	24	122
9	do	66	113
10	Semipalatinsk × Kansas Common	10	102

[1] Includes reciprocal cross.

progenies. The plants were self-pollinated to produce the F_2 populations. The 51 F_1's from the 10 crosses did not differ statistically, perhaps because of the small number of plants available, but there were highly significant differences among the pooled F_2 lines. Since calculations on reciprocal crosses did not show significant differences, it was possible to pool all of the F_2's from a cross thus greatly increasing population size. These F_2 populations exhibited significantly different levels of resistance, which meant that certain of the parent plants were transmitting different reaction tendencies.

A low, nonsignificant correlation between these F_1's and their F_2 progeny was noted in all cases except the one in which 26 F_1 plants were available and tested. The true means of the other F_1 plants probably were not secured since each was represented by only 1 to 7 plants, in contrast to the number of F_2 plants, which ranged from 10 to 126 plants in a population. Undoubtedly the larger numbers in the F_2 generation gave a better estimate of a cross than the mean of the F_1's.

Several crosses and backcrosses among inbred lines were made in the summer of 1941. Eleven had progenies of 11 or more. In table 5 the 11 crosses are listed and infection indices of the F_1 plants are given. Analysis of variance showed that highly significant differences occurred among the crosses of the inbred plants. This was not true of the crosses among the open-pollinated plants reported above.

TABLE 5.—*Means of infection for crosses of inbreds and backcrosses*

Parent varieties	Lines crossed	Number of plants in cross	Mean of infection
(Semipalatinsk × Ladak) × Ladak	7– × F	32	80.7
Kansas Common	C3 × C4	36	86.7
Do	B2 × C3	11	89.6
Do	B8 × C5	14	94.2
Kansas Common × Hairy Peruvian	C3 × D2	11	96.8
Kansas Common	B8 × C4	15	96.9
Do	B2 × B8	40	99.4
Do	C4 × C5	31	103.0
(Ladak × Hairy Peruvian) × Hairy Peruvian	6– × D	31	105.8
(Hairy Peruvian × Kansas Common) × Kansas Common	3– × B	11	111.3
Hairy Peruvian × (Kansas Common × Hairy Peruvian)	D × 5–	12	112.8

The larger numbers present in the crosses of S_1's and backcrosses, the more nearly homozygous constitution of the parents, and the closer selection of parents probably accounted for these different results rather than inbreeding itself. The F_2 population from the crosses among inbred plants was not tested.

EFFECT OF INBREEDING AND SELECTION IN INBRED LINES

The distribution of plant indices of the first and second inbred generations of a resistant Kansas Common plant, B, was studied. The mean of the S_1 generation was 102 with a standard deviation of 37.9. The average score of the original P_1 plant B was 100, which is the result from approximately 10 inoculations, as several cuttings were available. Thus, this plant transmitted resistance to its offspring approximately at its own reaction level. The plant B2, a selection

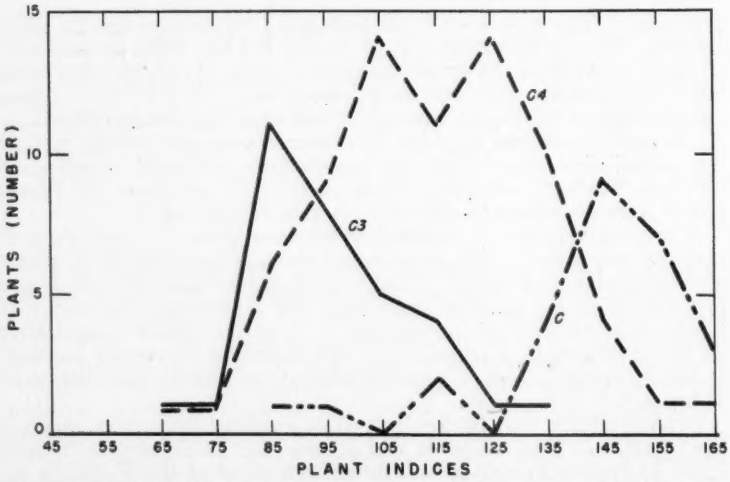

FIGURE 2.—Black stem indices for the first and second generation inbred progenies from the Kansas Common variety of alfalfa. Line C represents the S_1 generation and lines C3 and C4 the S_2 generation. Significant increases in resistance were obtained.

from the selfed progeny of B, scored an average of 93. Selfed progeny of B2 had a mean of 95 with a standard deviation of 21.4. Thus B2 transmitted similarly to B. The progenies of B and B2 did not differ statistically.

Figure 2 shows the distribution of plant indices of the S_1 and S_2 generations of a susceptible Kansas Common plant, C. It is readily seen from the graph that this plant produced mostly susceptible offspring, as only 2 plants scored less than 100. The mean of the 27 selfed progenies was 147 with a standard deviation of 29.0. Plant C3 had an average score of 110, and the average of its 32 selfed progenies was 96.1 with a standard deviation of 14.1. Plant C4, a sister of C3, averaged 116, and its 72 selfed progenies averaged 115 with a standard deviation of 19.6. Analysis of variance showed that all 3 progenies differed from each other significantly. Thus selection raised the resistance of this line considerably above the original level.

Figure 3 shows the distribution of the S_1 and S_2 generation plant indices for a selection from the Hairy Peruvian variety. Its selfed progeny averaged 140, having a standard deviation of 32.6. D2, a

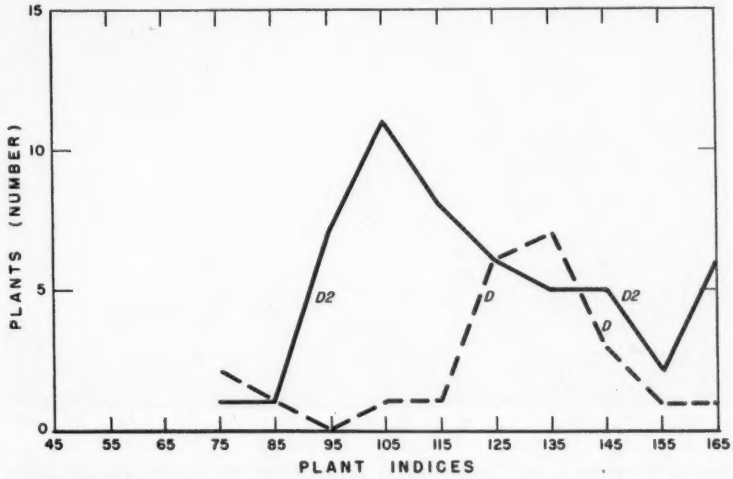

FIGURE 3.—Black stem indices for the S_1 and S_2 generations of Hairy Peruvian alfalfa. Line D represents the first inbred generation and line D2 the progeny from a resistant selection.

selection from the S_1 progeny scored 117 and the mean of its progeny was 117. Analysis of variance showed that the progeny of D2 averaged significantly more resistant than the progeny of D.

The plant indices for the S_1 and for the unselected S_2 generations from a Ladak plant, H, were studied. The S_2 generation was an unselected population, as a number of S_1's were selfed and the seed mixed. The mean of the S_1 generation was 121 with a standard deviation of 32. The mean of the S_2 generation was 131 with a standard deviation of 23.1. The difference between the two means was not significant. Thus in this case there was no selection and the

S_2 generation was not quite as resistant as the S_1, although the difference was not significant.

These four cases illustrate the value of selecting for resistance to black stem in an inbreeding program on heterozygous material. In all cases the selection of resistant plants in the S_1 generation tended to produce more resistant plants in the S_2 generation than in the S_1, while in the case of an unselected population there was no change in resistance in the S_2 generation as compared with the S_1.

EFFECT OF SELECTION IN HYBRID LINES

Figure 4 shows the distribution of plant indices of two F_2 lines, 3 and 7, derived from crossing open-pollinated plants. Line 3 resulted from crossing a susceptible Hairy Peruvian with a resistant Kansas Common. Line 7 was the cross of a resistant Semipalatinsk by a resistant Ladak. Crosses of two susceptible plants, Ladak by Hairy Peruvian, gave about the same distribution as shown by line 3. The

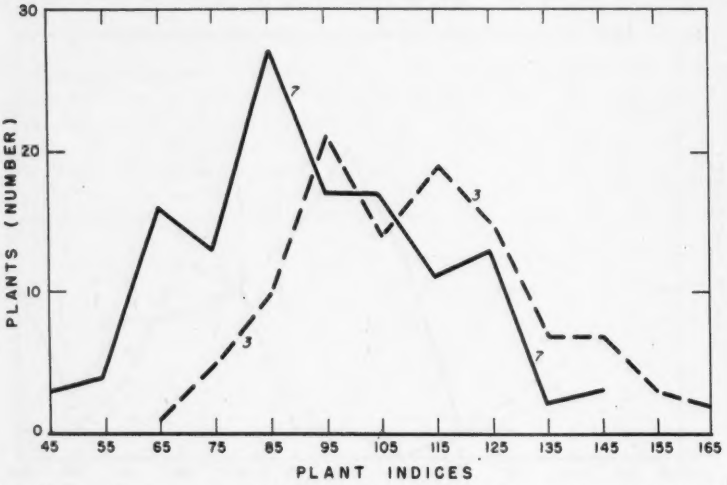

FIGURE 4.—Black stem indices for F_2 plants from crosses of open-pollinated plants. Line 3 represents a cross of susceptible Hairy Peruvian by a resistant Kansas Common and line 7 a cross of resistant Semipalatinsk by resistant Ladak.

means of the F_1's of the three crosses did not differ significantly, but those of the F_2's did. Line 7 was significantly more resistant than the other lines. Thus the cross involving two resistant plants produced more resistant offspring than either of the other two crosses, one of which had one susceptible parent and the other two susceptible parents.

These results corroborate those from crosses of the inbreds. The progeny carried a high degree of resistance if the parents were resistant, and proved susceptible if the parents were susceptible.

SELECTION OF HIGHLY RESISTANT PLANTS

One plant from one of the Kansas Common wilt-resistant selections proved to be highly resistant to black stem. On three successive inoculations it scored 43, 41, and 56 as compared with an average of 82 for the most resistant line included in the same tests. On one inoculation in the fall of 1942 this selection scored 69. A comparison of this plant with Hairy Peruvian is shown in figure 5. Another plant from the F_2 generation of a Semipalatinsk by Ladak cross averaged 48 for two inoculations.

FIGURE 5.—An illustration of extreme resistance to black stem in a selection of Kansas Common alfalfa (*A*), and of susceptibility in a plant of Hairy Peruvian (*B*).

PREDICTION OF THE REACTION OF THE F_1 GENERATION PLANTS

It is generally accepted that the average of the selfed progeny from a plant is a better index of a plant's transmitting ability than the actual score of that plant. For this reason the means of the selfed progeny of each parent in the crosses listed in table 5 were averaged to obtain an expected F_1 index of infection. The correlation of the means of the F_1's and the expected indices was significant at the 5-percent level. This indicated that the F_1's were intermediate between the parental transmitting abilities as measured by the reaction of inbred progeny from the parent plants.

DISCUSSION

These studies showed that inheritance of resistance to black stem was definite but not simple. Intergrading classes of resistance were observed in all populations. Immunity was not observed in any plants. Much variability was found to exist within open-pollinated varieties, among hybrids, and in the selfed progeny from one-generation inbreds. Slight deviations in laboratory conditions resulted in marked differences in infection.

The numerous sources of variation made extensive use of statistical analysis mandatory if a clear view of the results was to be obtained and positive evidence of inheritance revealed. The fact of inheritance of resistance was shown in tests of varieties, various kinds of selections, and in the progeny of hybrid combinations. Even the reactions of the F_1's from crosses of inbreds and backcrosses could be predicted by calculating indices from the reaction of the selfed progeny of the parents. Complete dominance of resistance or susceptibility was lacking except in one case, when dominance of resistance was noted. If a large number of favors and different mechanisms were involved in the inheritance of resistance, then resistance might be both dominant for some genes and recessive for others.

The exact nature of the mild type of resistance observed in these studies is not understood, but it must be either morphological or physiological. In respect to the former, it should be noted that a glossy, hairless leaf surface common in Ladak made thorough wetting of the leaves more difficult than in Hairy Peruvian where the leaf surface is covered with epidermal hairs. This would suggest an escape mechanism. In an effort to discover a physiological factor, an experiment was conducted to test the cell sap extracted from resistant and susceptible plants. The respective saps were sterilized in Erlenmeyer flasks; then the fungus was transferred to the sap and allowed to grow. The resultant mycelial pad weights were compared. There was no difference. These results, while negative, do not prove that living protoplasms may not have differential responses to the fungus.

The question of physiologic races of the fungus was investigated. Diverse collections of the fungus were isolated which showed consistently different growth characteristics on artificial media in the laboratory. Acidified oatmeal agar proved to be the most effective medium for differentiating the collections. Single-spore isolates within a collection gave little or no variation in growth characteristics. Tests of these collections on clonally propagated alfalfa plants showed that strain No. 7 was significantly more virulent than either a mixture of the eight strains or any of the other seven strains individually. This virulence was not correlated with colony growth. The index of infection was 138 for strain No. 7 and 119 for the mixture of all eight strains. Lowest infection was 99 for strain No. 1. Thus strain differences were established. However, in the tests reported here it is considered that this was not an important source of variability.

Resistant selections were obtained among inbred plants derived from certain open-pollinated varieties and hybrid populations and as inbreeding proceeded, variability of reaction within a line declined. Paralleling this means of isolating resistant plants was the success achieved by selecting for resistance without resorting to inbreeding.

Since most of this investigation involved inbreeding, equal numbers of inbred and open-pollinated plants were not studied. In spite of this, there was evidence, as already pointed out, that the highest levels of resistance achieved from selecting among inbreds were matched by selections in open-pollinated stocks. Self-sterility, low vigor of inbred lines, and the labor involved reduces the value of inbreeding in an alfalfa improvement program. It would seem easier to secure natural crosses in a polycross nursery composed of selected plants and, by careful selection among the progeny thus produced, raise the level of resistance.

Several other leaf spot diseases of alfalfa are important and may be confused with black stem both in the symptoms of the disease and the damage done to the plant. Hence in a breeding program it is important that resistance to all similar diseases be observed. Greenhouse testing with pure cultures of the fungus could be used to advantage in making specific tests for resistance.

SUMMARY

This paper presents a study of the reaction of alfalfa varieties and strains to the black stem disease caused by *Ascochyta imperfecta* Peck. The fungus affects the stems and leaves of the plants, causing reduced yield and quality of the crop.

Different varieties of alfalfa showed different levels of infection in the field and in the greenhouse.

Inbred progeny from resistant and susceptible plant selections showed inter- and intravarietal differences.

There was a significant correlation of resistance between S_1 and S_2 lines from five varieties of alfalfa. Variability was lowest in the S_2 generation.

Crosses among open-pollinated plants reacted similarly in F_1, perhaps because of the small number of plants available, but significant differences were found among lines in the F_2 generation.

Crosses among inbreds and backcrosses showed significant differences.

The F_1's tended to be intermediate between the parents, although in a few cases a tendency toward dominance of resistance was noted.

Inbreeding followed by selection and hybridization followed by selection in F_2 proved to be valuable in raising resistance levels.

The fact that inheritance conditions the resistance of alfalfa plants to black stem was amply demonstrated, but the factors responsible for the inheritance of resistance were not determined. One plant characteristic which might possibly influence resistance to black stem was noted; namely, the glossy, hairless leaf surface in Ladak which made thorough wetting of the leaves difficult.

Physiological races of the fungus were observed in a study of the growth characteristics of different collections on artificial media. These races showed significantly different virulence on alfalfa plants.